STUDY OF HISTOLOGY OF MAMMARY GLAND-VARIOUS AGES

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THANJAVUR MEDICAL COLLEGE,

THANJAVUR

THE TAMILNADU DR.MGR MEDICAL UNIVERSITY,

CHENNAI

APRIL - 2015

CERTIFICATE

This is to certify that dissertation titled "**STUDY OF HISTOLOGY OF MAMMARY GLAND - VARIOUS AGES"** is a bonafide work done by **Dr.K.ARUNA** under my guidance and supervision in the Department of Anatomy, Thanjavur Medical College, Thanjavur during her post graduate course from **2012 to 2015.**

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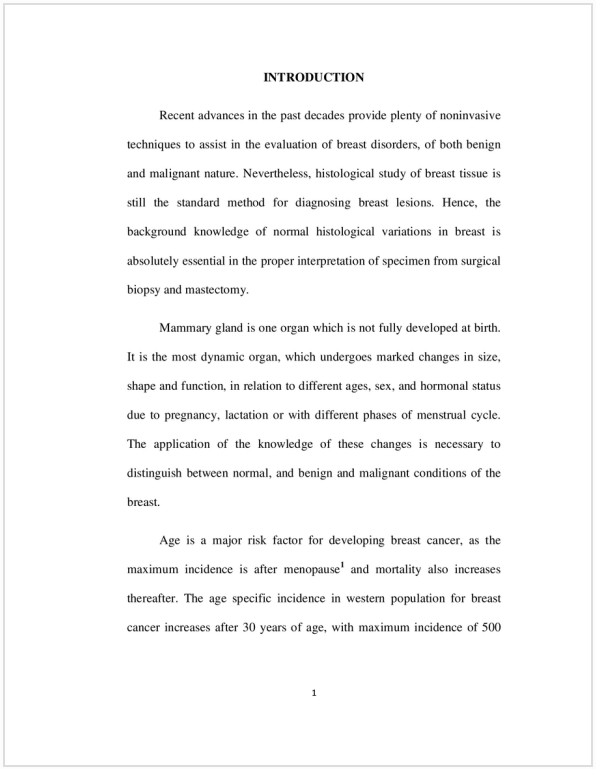

INTRODUCTION

Recent advances in the past decades provide plenty of noninvasive techniques to assist in the evaluation of breast disorders, of both benign and malignant nature. Nevertheless, histological study of breast tissue is still the standard method for diagnosing breast lesions. Hence, the background knowledge of normal histological variations in breast is absolutely essential in the proper interpretation of specimen from surgical biopsy and mastectomy.

Mammary gland is one organ which is not fully developed at birth. It is the most dynamic organ, which undergoes marked changes in size, shape and function, in relation to different ages, sex, and hormonal status due to pregnancy, lactation or with different phases of menstrual cycle. The application of the knowledge of these changes is necessary to distinguish between normal, and benign and malignant conditions of the breast.

Age is a major risk factor for developing breast cancer, as the maximum incidence is after menopause[1] and mortality also increases thereafter. The age specific incidence in western population for breast cancer increases after 30 years of age, with maximum incidence of 500

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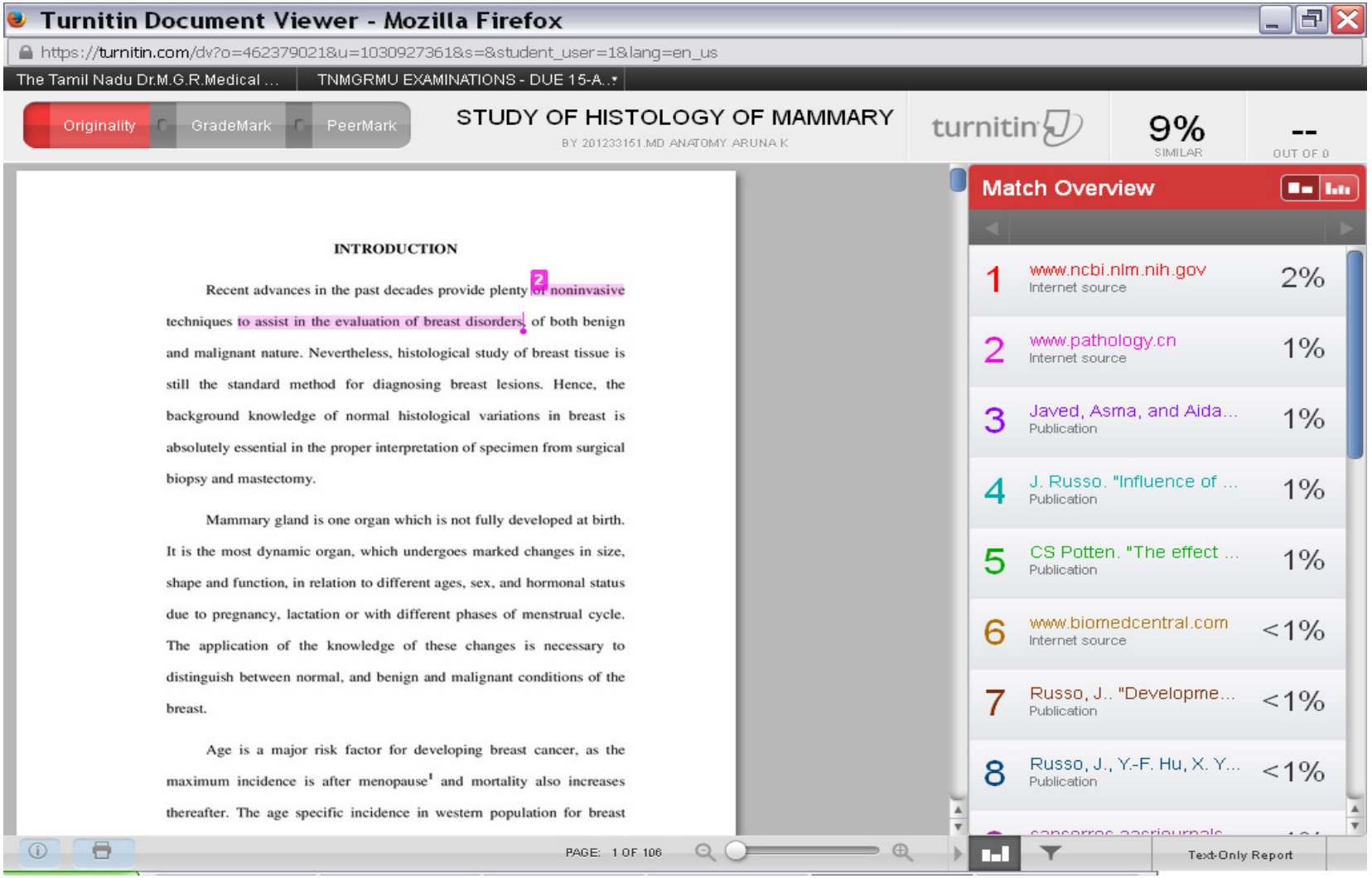
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I dedicate this work to

Lord SrimanNarayana

and

to my Guru

Her Holiness Sri SathguruGnanandaSaraswathi, the divine

force who guides me throughout my life towards the best.

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STUDY OF HISTOLOGY OF MAMMARY GLAND - VARIOUS AGES

Background

Breast cancer is one of the common carcinoma in women. As epidemiological studies point out, the risk of developing cancer is more in case of older (>50 years) and nulliparous women as compared to parous women.

Aim

To study the normal morphological changes with age and parity based on the lobular type; the changes in breast during menstrual cycle. Using immunohistochemical marker S100, the pattern and intensity of the myoepithelium in relation to age and lobular type; and its morphology during different phases of menstrual cycle were studied.

Material & Methods

A total of 36 normal breast tissues from surgical biopsies and mastectomy cases were taken up for study, using routine H&E staining. 11 samples with regular menstrual cycle and known LMP were taken to study changes during the menstrual cycle. 4 samples were used to study the myoepithelial changes using IHC with S100 marker.

Observation

The most common Type in women of < 20 & > 50 years and nulliparous women was Lobular type I. Type II was almost equally distributed in all ages except in midforties. Type III was seen mainly in reproductive age group and parous women. The concordance of chronological date with that of morphological phase was 72.7%.

IHC slides showed regular staining pattern in 20, 21, and 35 years of ages except in the slide of post -menopausal women (55 years), which showed irregular pattern. The intensity of the staining decreased with increasing age in the samples.

Conclusion

The lobular type I was the predominant type in breast tissue women over 50 years and in the nulliparous women, increasing the risk for developing carcinoma, as compared to parous women and may need frequent follow up to detect early stages of carcinoma. IHC study showed decreased intensity and irregularity of staining in older post-menopausal women which may be due to the age related morphological changes the myoepithelium.

INTRODUCTION

Recent advances in the past decades provide plenty of noninvasive techniques to assist in the evaluation of breast disorders, of both benign and malignant nature. Nevertheless, histological study of breast tissue is still the standard method for diagnosing breast lesions. Hence, the background knowledge of normal histological variations in breast is absolutely essential in the proper interpretation of specimen from surgical biopsy and mastectomy.

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Age is a major risk factor for developing breast cancer, as the maximum incidence is after menopause[1] and mortality also increases thereafter. The age specific incidence in western population for breast cancer increases after 30 years of age, with maximum incidence of 500

cases per one lakh women falling under the age group of sixty to seventy years.[1]

Studies have identified nulliparity as a risk factor for developing breast cancer[2]. Epidemiological studies show relative risk of breast cancer in nulliparous women, as compared with parous women, to range from 1.2 to 1.7[2,3,4] especially in the age group of forty to forty five years. Studies also show that the protective effect of pregnancy, from developing malignancy in the breast tissue, depends on two factors

a. Whether the pregnancy has reached full term or not. Incomplete pregnancy does not afford any protection to the breast tissue.

b. Age at first full term pregnancy. Maximum protective effect is seen when the first full term pregnancy occurs at an age of less than twenty years.[4, 5, 6]

The above two factors probably afford protection from breast cancer by increasing the degree of differentiation of the terminal duct lobular unit (TDLU) to maximum.

Animal studies show that a carcinogen affects only breast tissue with undifferentiated terminal duct structure and fails to induce cancer in completely differentiated breast tissue after full term pregnancy.[7, 8, 9]

Studies by Vogel *et al* (1981)[10] showed high mitotic activity in the breast tissue during the follicular phase of menstrual cycle, as an indicator of proliferation. But, studies by Longarce and Bartlow (1986),[11] Going *et al* (1988)[12] and others[13,14] reported maximum proliferative activity in the breast tissue in the secretory phase of menstrual cycle.

Mauvais Jarvis *et al (*1986) states that, estrogen acts as a major growth promoting hormone for the breast tissue. And in some instances, progesterone acts as anti-estrogen and inhibits estrogen induced growth and differentiation of breast tissue.[15]

Korenmann (1980) proposes that the risk of cancer breast may be due to defective secretory phase with low or absent levels of progesterone. He also states that low age at menarche as a risk factor for developing breast malignancy due to increased incidence of anovulatory cycles in these women, which in turn leads to unopposed action of estrogen.

Studies as early as in 1934 by Dawson, [16] by Welling *et al* [17]in subgross and microscopic study of tissue from breast carcinoma, and other authors[13, 18] indicated that the understanding of the architecture of normal breast, its level of differentiation is absolutely essential, as they might be responsible for initiation of tumor in the undifferentiated breast

tissue, and thereby improving the sensitivity of the histological study as a mode of standard diagnostic method.

Features common to all studies regarding the morphology of human breast tissue is variability of result from sample to sample. The reason for this variability remains unclear.[19]

May be more data is needed to be collected by many more groups to analyze the histological features of human breast tissue, in different age groups, in different phase of menstrual cycle and in relation with parity. Hence, attempt has been made to study these changes in the normal tissue from surgical open biopsy, done for benign breast disease and in tissues from mastectomy specimens.

AIMS AND OBJECTIVES

1. To study and record the histological features of normal breast tissue components in relation to age and parity.

2. To study and record changes in the normal breast tissue during different phases of menstrual cycle.

3. To demonstrate myoepithelial cell staining pattern and intensity in premenopausal & postmenopausal age group,lobular types and during the luteal phase of menstrual cycle using Immunohistochemistry marker (IHC) - S100.

REVIEW OF LITERATURE

Historical Review of Mammary Gland

Mammary gland in Latin means “glandula mammaria”. Mammals get their name from the word mammary. In humans, fully developed mammary gland is situated in the breast, which protrudes constantly and is relatively large for body size. In other mammals, glands protrude only when filled with milk. The position and number of glands widely vary in each mammal. The mammary glands of mammals other than primates are called dugs.

In general, in most mammals, mammary glands develop along its mammary ridge in pairs. The Nipples vary in number from 2, as in most primates, to 18 as in pigs. Very few mammals have nipples in odd numbers. E.g.: Virginia opossum.[20] Male mammals have mammary gland and nipple which is rudimentary, with very little exception. Male mice do not have nipples[21] and male s horse do not have both mammary gland and nipple. Male Dayak fruit bat has mammary gland, which is lactating.

Mammary glands are used as factories producing proteins. Many laboratories produce protein for pharmaceutical use mammary gland from transgenic animals, mainly cows and goat.[22] Complex glycoproteins like anti-thrombin and monoclonal antibodies, which cannot be engineered genetically from bacteria, are produced from live mammals, which is much cheaper than cell cultures.

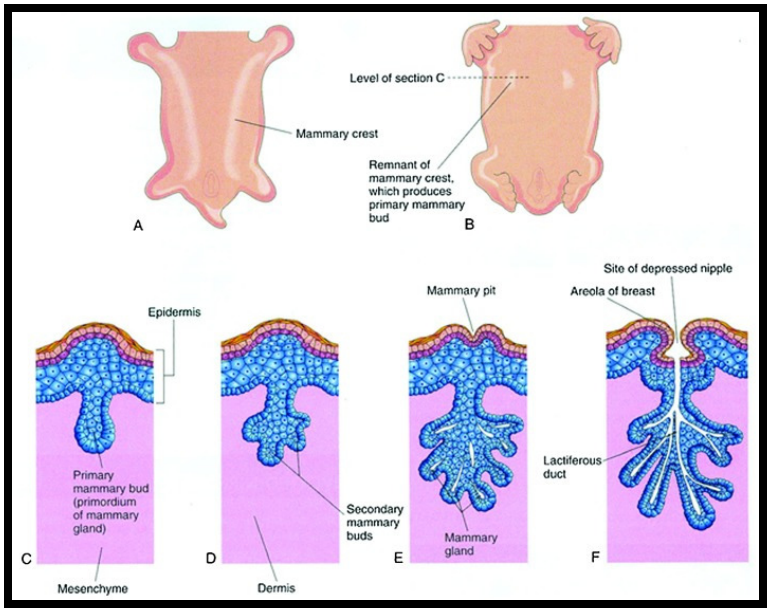
As mammary gland does not fossilize, its evolutionary theories cannot be proved. Many current theories are comparisons between different living mammals. These theories proposed that the gland from early mammals, which are used either for keeping the egg moist,[23] or to prevent infection[24] or to directly hatch the eggs have evolved into mammary gland.

DEVELOPMENT OF MAMMARY GLAND

Prenatal & Perinatal Development

Mammary gland consists of parenchyma and stroma, which develops from ectoderm and mesoderm respectively. The human breast houses mammary gland that shows extensive tree like networking of branching ducts. They show plasticity and remodeling in adulthood, the factor which increases the susceptibility to carcinogens. Congenital and acquired disorders of breast often have a basis in development.

Fig - 1 Development of Mammary Gland[25]



Prior to puberty, development of breast is the same in both sex. The mammary glands begin to develop in fifth to sixth week of gestation, with the formation of the bilateral mammary ridges extending from the

axilla to the groin .[26,27] Except for the placodes at the level of fourth intercostal space, most of the ridges do not develop and disappears.

At the early stages, development is largely independent of the influence of sex steroid hormones. At fifteenth week of gestation, the breast is sensitive to testosterone, triggering formation of breast bud.

Table - 1 Stages of Prenatal development of the Human Breast (Russo and Russo[29])

Stage	Mammary gland development	Size
	Embryonic stages	
1	Ridge	<5 mm embryo
2	Milk hill	>5.5-mm embryo
3	Mammary disc	10–11 mm embryo
4	lobule type	11.0–25.0-mm
5	Cone	25–30-mm
6	Budding	30–68-mm
7	Indentation	68-mm to10 cm
	Fetal stages	
8	Branching	10 cm
9	Canalization	Gestation - 20 to 32 weeks
10	End-vesicle - single layer of cells filled with colostrum secretion	Newborn

Eventually, epithelial columns develop within the mesenchyme of the breast bud, giving rise to the lobules. The fetal papillary dermis surround the developing cords and gives rise to fibrous connective tissue.

Portions of this mesenchyme differentiate into fatty tissue by 20 to 32 weeks. Myoepithelial cells also develop within the period of 23 to 28 weeks.[26]

In the last few weeks, the cords undergo branching and canalization. And at the time of birth, epidermis invaginates to form lactiferous ducts and evaginates the mammary pit to forms nipple.

Also in the last few weeks of gestation, the mammary gland becomes responsive to steroid hormones of maternal and placental origin, causing secretory changes in the developing gland. On withdrawal of maternal and placental sex hormones at the time of birth, prolactin causes colostrum secretion, and enlarged and palpable breast buds in both male and female neonates.

When the level of prolactin decreases, secretory activity declines, and glands regress and remains inactive till puberty[26, 27]. At this stage, the breast may show formation of lactiferous ducts with very few branching without any alveolar differentiation and attempt to form rudimentary lobular structure.

Postnatal Development

The first two years of life is important as far as breast maturation and involution is concerned. The gland remains quiescent from 2 years to puberty. Anbazhagan *et al*[28] has described well accepted stages in morphology and functions of human breast from birth up to 2 years of age.

Morphological and Functional changes in Infant Breast[28]

The morphological changes begin in the neonatal period with 3 different types. Functional changes (four stages) show linear progression from birth to 2 years, with periods of apocrine metaplasia (stage II-III) before stage III (involution).

Morphological changes in Infant breast

Type I Ductal branching - 0 to less than 2, Dichotomous.

Type II Ductal branching - > 2 Dichotomous. No Terminal Duct Lobular Unit (TDLU) seen.

Type III Ductal branching - more. Well-developed lobules seen.

Functional changes in Infant breast

Type I Ductal System - lined by secretory type of epithelium.

Type II Ductal System - lined by secretory and apocrine type epithelium.

Type III Ductal System - lined by apocrine type of epithelium.

Type IV Ductal System - lined by apocrine type of epithelium; Involuting ducts- lined by multilayered epithelium.

Morphological types do not show this linear progression. The breast development can have any number of variations with the different morphological types and functional stages. The time from regression of breast tissue to the stage of very small ducts with fibrous stroma varies, but usually seen at the end of infancy.

At Puberty

Female breast

The adolescent period begins with the first sign of sexual change at puberty and terminates with sexual maturity.[30, 31] In females, thelarche is the first secondary sexual character to appear preceding pubarche by 6 months.[25] The rudimentary pubertal breast show both glandular and stromal growth. The glandular tissue show elongation and branching of

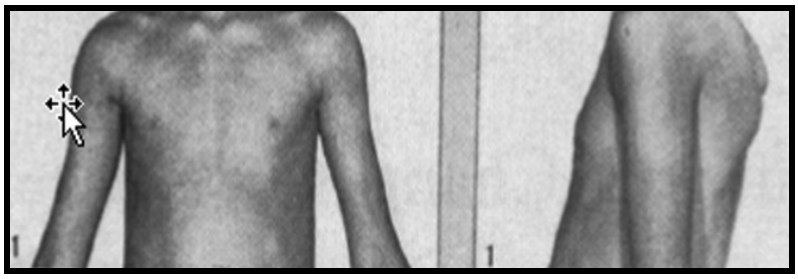
the duct forming Terminal Duct Lobular Unit (TDLU), but they do not show any secretary changes at this stage. In comparison, rodent mammary gland, the secretary changes unit (TDLC) they do not, show any secretary changes until pregnancy.

Though the immediate stimuli for mammary gland development are estrogen, it depends on growth hormone secreted by pituitary gland to stimulate the synthesis of Insulin like growth factor in the gland.[25] The age range for this is 8 ½ to 13 ½ years. Absence of any development of breast beyond this age should be investigated.

Tanner[25] described the most well accepted microscopic stages of breast development at puberty (Thelarche). He described five characteristic stages. Gross anatomical changes starts with

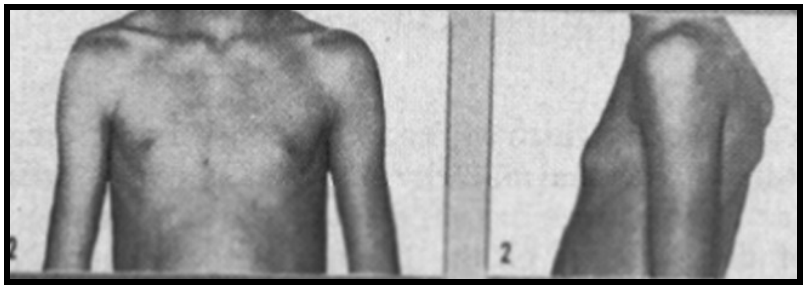
Stage - I: Small elevated nipples; No palpable breast tissue, as there is no additional development of stroma or parenchyma.

Fig - 2a Tanner's stage I



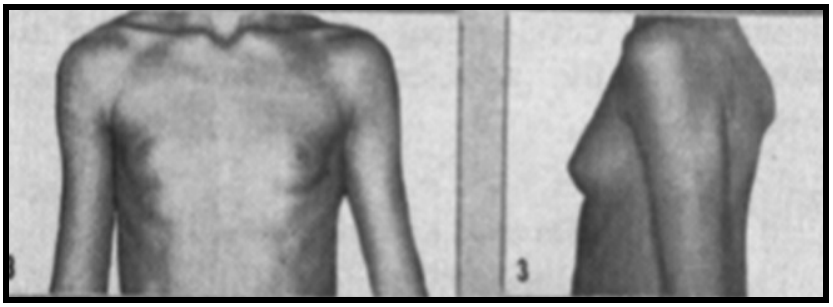
Stage - II: Formation of breast bud with elevated nipple; small amount of breast tissue with enlargement of areola. The average age of girls in British population occur 6 months earlier compared to United States population. Some literatures point out earlier age of onset in United States population of adolescent girls on an average 9.8 vs.10.8 between individuals of same age. Significant difference is also seen in breast development based on hormonal concentration, level of maturation and ethnicity.

Fig - 2b **Tanner's stage II**



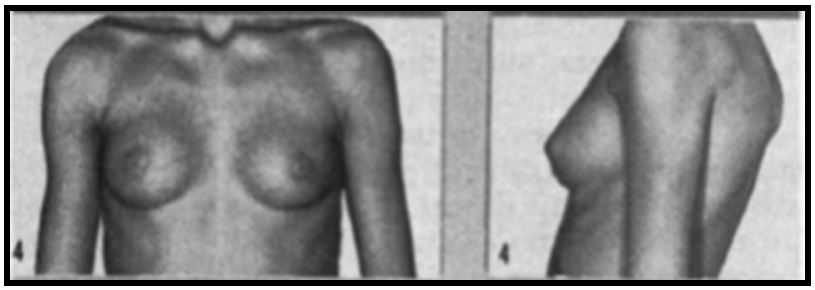
Stage 3: Further enlargement of breast and areola seen. No separate contour is noted in this stage. The average age at this stage is 12.5 years.

Fig - 2c **Tanner's stage III**



Stage 4: Enlargement of nipple and areola. Formation of secondary mound on the breast. The average age is 13-14 years for this stage. Some girls progress from stage 3 to 5 without having stage 4.Menarche usually occurs between stage 4 and 5.

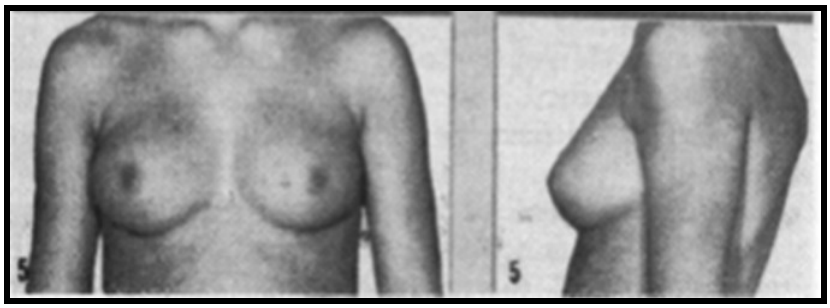
Fig - 2d **Tanner's stage IV**



Stage 5: Recession of areola with resulting loss of contour seen.

Represents mature breast. Average age is 15 years.

Fig - 2e **Tanner's stage V**



The average time between stage 2 to 5 is 4 -4 ½ years. Breast shrinkage after or during these stage of development may occur due to loss of fatty tissue. This is particularly relevant in cases of Anorexia

Nervosa leading to wrinkled appearance to the breast - Instant senility, the term coined by Capraro and Dewhurst. Significant development of the nipple takes place with increase in size and diameter of the nipple. During puberty, there is increase from 5 to 6 mm between stages 1 to 5.

Male breast

In pubertal male breast, no further development takes place due to increasing levels of testosterone. About 40% of the pubertal male may develop gynecomastia, temporarily possibly due to relative high level of estrogen. In gynecomastia, enlargement of breast is not due to lobular hypertrophy but ductal and stromal hypertrophy.[25] Boys also can have increase in size of nipple during puberty.

GROSS ANATOMY

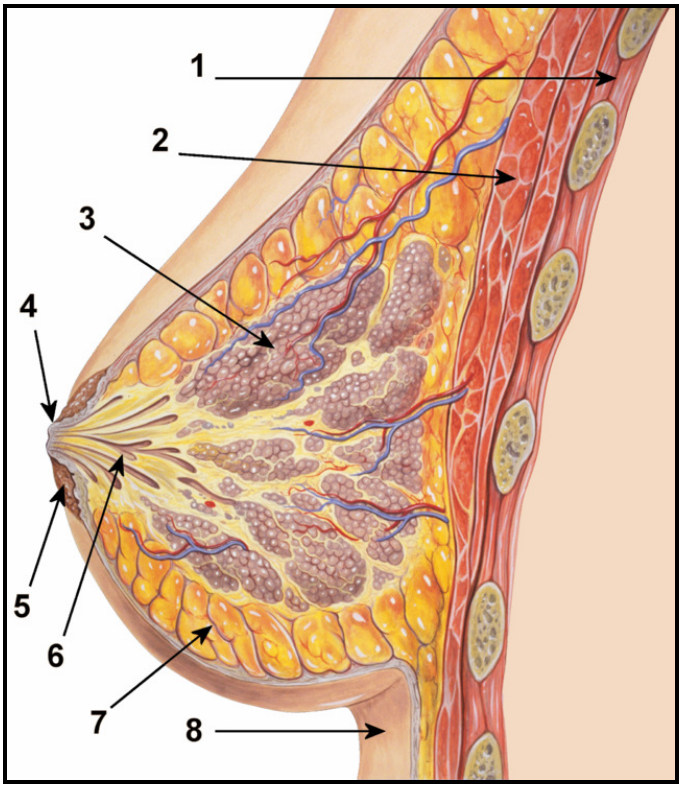
Adult Female breast

Breast is a modified appendage of skin, situated in the anterior aspect of chest wall. It lies in the superficial fascia, over the Pectoralis major muscle. Occasionally, breast tissue may extend into the axilla or even the Pectoralis muscle as accessory breast tissue.[32]

Gross anatomy of an adult female breast is made of ducts, ductules and lobulo acinar units, embedded in fibrous and fatty connective tissue. The amount and the proportion of fibrous and fatty components vary with age and built of the individual.

At puberty, the connective tissue stroma is very dense and fibrous, which gradually becomes fattier, as age advances.[26, 33] The duct lobular system is arranged in segments which are not well defined. There is no obvious anatomical boundary to separate each segments.[26]

Fig - 3 Gross anatomy of Adult Breast



1. Chest wall
2. Pectoralis major muscle
3. Lobule
4. Nipple
5. Areola
6. Lactiferous duct
7. Adipose tissue
8. Skin

The resting (Non pregnant and non-lactating) breast consists of ducts radiating from the nipple and glandular parenchyma called the lobules.

They make up relatively small portion of the breast. These structures are surrounded by a stroma made of fibrous connective tissue and fat. These stromal components vary between individuals and with age.[34] Collagenous bands connect the fibrous tissue to the skin, called Sir Ashley Cooper's suspensory ligament, which lacks the dense and parallel collagen fibers as seen in true ligaments. The increase in the laxity of this ligament may lead to breast ptosis.[34]

The duct glandular tissue is organized into ducts and lobules. It has multiple central ducts converging towards the nipple surface. The number of ducts opening into the nipple is usually 15 to 20. But, the number of ducts that can eject milk during lactation or can be cannulated in typically 6 to 10 in number. The explanation may possibly be due to branching of ducts in the nipple sharing single ostium by more than one duct or due to blind ducts which do not lead to any glandular tissue.

Before opening into the nipple, the ducts show dilation called lactiferous sinuses which are lined by stratified squamous epithelium as seen microscopically and are filled with keratin. The sharp transition between the double layered ductal epithelium to that of stratified squamous epithelium forms the basis for the lack of extension of intra ductal carcinomas into the nipple. Only Ductal Carcinoma in Situ (DCIS) associated with Paget's disease of nipple can spread to the nipple.[34]

The study of the lobular architecture of breast tissue is very challenging, as the ducts and branching systems are not easily discernable by mere inspection. In vivo injection studies (ductography) or injection studies in surgical and autopsy specimens are very difficult. Better knowledge of the lobar anatomy will be very helpful for understanding normal development of breast and the spread of pre invasive malignant cell in the DCIS or lobular carcinoma in situ.

Blood supply[34]

Medially, the breast is supplied by the internal thoracic artery, which gives perforating branches to the breast (Second to fourth intercostal spaces). Laterally the lateral thoracic and the thoraco acromion artery and posterior intercostal arteries. Venous drainage is through the tributaries of Axillary vein, second to sixth Posterior intercostal veins and also internal thoracic vein from the medial part.

Lymphatic Drainage[34]

The drainage of lymphatic show two main sites, axillary group and internal mammary group of lymph nodes. A quantitative study shows 75% of lymph goes to the axillary lymph nodes of the same side.

Significant lymph also drains into the internal mammary group, involving the medial as well as lateral parts of the breast. Minor drainage occurs to supra clavicular nodes, opposite side internal mammary group of nodes and posterior intercostal nodes.

Using lymph scintigraphy, Turner, Warwick and others challenged Sappey's belief that axillary drainage of lymph is through sub areolar lymphatic plexus. They stated that, a small number of lymphatics drain directly to the sentinel node, axillary node or internal mammary node without passing the sub areolar plexus. These anatomical considerations influenced the mode of sentinel node identification. Sentinel nodes are better identified by peritumoral injection (one third of cases) than by intradermal or subcutaneous tracer injections.

Innervation[34]

It is supplied by the lateral cutaneous branches of second to seventh intercostal nerves, along the mid axillary line and medially by the anterior cutaneous branch of 1st to 6th intercostal nerves. The nipple areola complex is rich in tactile Meissners receptor.

Adult male breast[26]

Adult male breast is composed of nipple and undifferentiated and rudimentary ductal system with both epithelial and stromal components. The stroma shows collagen and adipose tissue. But, in contrast to female breast, they lack lobule formation.

The breast in adult, and also adolescent male, can undergoes hypertrophic change - Gynecomastia, due to increased estrogenic stimulation. They resolve on its own, usually around 6-18 months, but it sometimes persists for 2 years.[25]

The nipple areolar complex lack pilo-sebaceous unit and hair, except in the periphery of areola, and the dermis of nipple shows numerous sebaceous glands. The tubercle of Montgomery is the representation of sebaceous gland and it is associated with lactiferous duct.[26]Sometimes, intra mammary lymph node is seen.[35, 36]

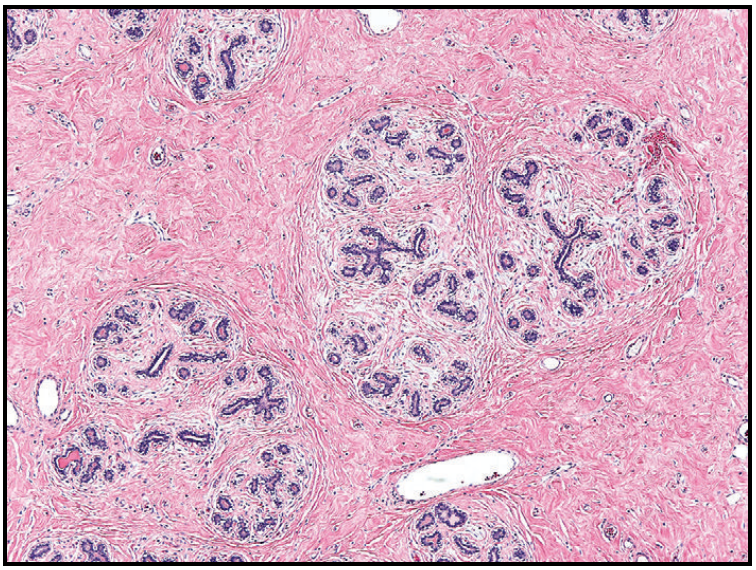
NORMAL HISTOLOGY

Breast in Puberty

The ducts in the pubertal female breast show elongation and branching. In the branching ducts, the lining epithelium shows thickening due to the influence of estrogen. It also increases periductal connective tissue density. The stroma also shows deposition of adipose tissue.[25] The duct branches partly dichotomously and partly sympodially. The branching ducts form terminal ductules or acini.

The collection of acini from one terminal duct with its intra lobular stroma is called Terminal Duct Lobular unit (TDLU). TDLU is the structural and functional unit of mammary gland.

Fig - 4 Histology of Pre pubertal Breast



Pre pubertal breast- shows Terminal Duct Lobular Unit (TDLU) with minimal branching of ducts. No acini are seen.

The growth and branching of ducts are not influenced by progesterone, but during ovulatory cycle progesterone causes the lobulo acinar and stromal growth. Even though, majority of development occurs are puberty, it continues well up to 35 years of age. But, terminal differentiation (lobular Type III and Type IV) [36] occurs only after full term pregnancy and lactation. The dividing ducts forms club shaped Terminal End Bud (TEB), at the epithelial-stromal boundary. These TEB bifurcates into smaller ductules or alveolar buds. As differentiation advances, alveolar buds further branch to become smaller and more numerous ductules. The lobular architecture in pubertal female breast show, predominantly, lobular Type I (Russo *et al*[36]) with an average of 11 alveolar buds or ductules around a terminal duct. This lobule is otherwise called virginal lobule.

The terminal ductules show double layered epithelial lining, while terminal end buds are lined up to four layers. Lobule formation starts within one to two years of menarche. Thereafter, the development varies from women to women in gradual manner over many years.

Adolescent male breast is composed varying amount of fibro-adipose stroma and normal glandular tissue with the ducts showing cuboidal lining.[26] Sometimes, the ducts may show pseudo papillary

proliferation in the epithelium with hyper chromatic nuclei, lobules will be absent and the surrounding fibrous stroma may show myxoid changes.

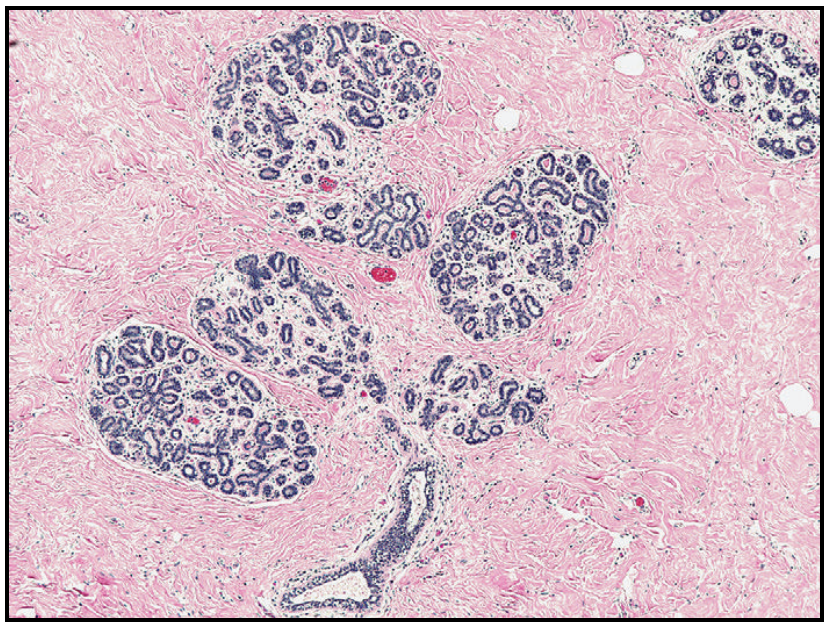
Adult Resting Breast

The importance of understanding and recognition of normal histological architecture of the duct-lobular system of adult breast cannot be over emphasized, since it is one of the main guides to distinguish between benign and malignant breast lesion[37]. In addition, the knowledge of normal structure gives information about various breast pathologies.

The hallmark of terminal duct lobular system is its double layered epithelium. It consists of inner luminal epithelial layer which has secretary function and outer basal or myoepithelial layer, having contractile function to push out the secretion towards the central duct.

Histologically, the inner luminal layer is made of cuboidal or columnar cells with pale eosinophilic cytoplasm, and somewhat uniform and oval nuclei. The basal myoepithelial cell layer is often inconspicuous, but may become conspicuous, during the the luteal phase with clear cytoplasm and prominent vacuoles (Phase II & IV).

Fig - 5 Histology of Adult Resting Breast



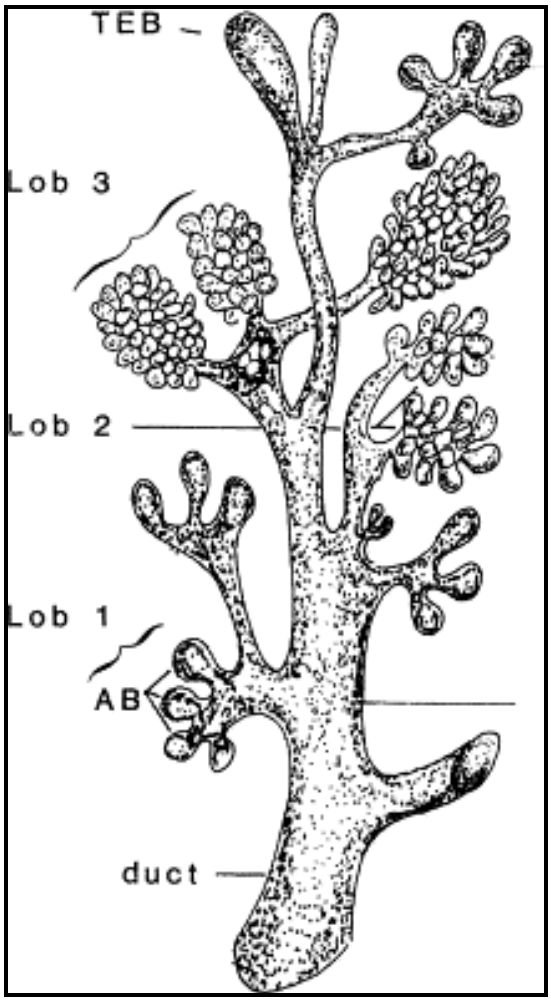
The normal adult female breast- well developed TDLU with extra lobular ducts dense fibrous connective tissue stroma (low-power view).

Outer to the myoepithelium is the basal lamina which is composed of Type IV collagen and laminin. The lobular arrangements of acini are surrounded by intra lobular stroma made of fibrous tissue and blood vessels. They also show variable number and type of inflammatory mononuclear cells like, lymphocytes, plasma cells and macrophages.

The inter lobular stroma varies from intra lobular stroma in having increased amount of dense collagen bundles, decreased number of cells and in addition has adipocytes.

The overall size of lobule and the number of acini or ductules are variable, as noted by Russo *et a.l*[3]. They have described four general lobular types in whole mount section of breast.[38, 39]

Fig -6 Lobular Types of Breast (Russo & Russo[29])



TEB - Terminal End Bud
Lob 2 - Lobular type 2
Lob 1 - Lobular type 1
Lob 3 - Lobular type 3
AB – Alveolar bud
Duct – Extra lobular duct

Lobular Type I:

- They are mostly rudimentary and are made of ducts with sprouting alveolar buds or the terminal end buds **(TEB)**.
- They are mostly seen is pre pubertal and nulliparous women of all age groups. With 60%-85% of the lobules belonging to this type.
- The average number of alveolar buds or ductules per lobule is approximately **11 ductules / lobule**.

Lobular Type II:

- This type shows addition al development of alveolar bud.
- The average number of alveolar buds or ductules per lobule is approximately **47 ductules /lobule**.

Lobular Type III:

- This type shows more number of ductules or alveolar buds as compared to type II.
- This type is seen predominantly in parous women and peri menopausal women.
- The average no of ductules per lobule is approximately **80 ductules / lobule**.

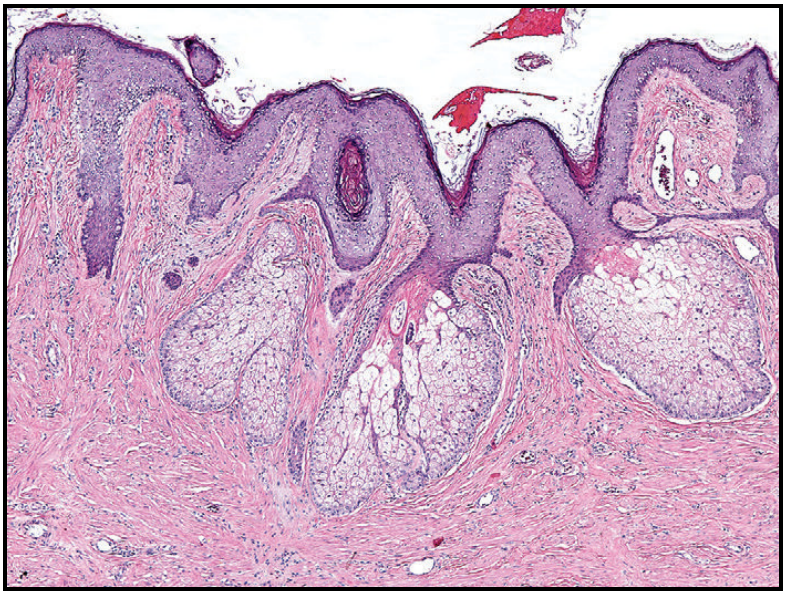
Lobular Type IV:

- These are the most differentiated type of ductules.
- They are seen during pregnancy and lactation.

Nipple-Areolar complex

The histology of nipple and areola show stratified squamous epithelium extending up to the lactiferous sinus. Some of the nipple epithelium may show cells with clear or pale cytoplasm, occasionally called Toker cells. They are benign as compared to cells seen in Paget cells, which are malignant.[35, 40] Toker cells are found is 11% of normal nipples[26]. Sometimes they are also seen along the milk line structure, like accessory nipples.

Fig – 7 Histology of Nipple



Nipple shows keratinized squamous epithelium, sebaceous glands, and ducts (Low-power view).

The part of the ducts near the nipple typically shows pleated or serrated contour. They are surrounded by a stroma rich in circular and longitudinal smooth muscle bundles, collagen and elastic fibers. Occasionally, lobules may be seen in the nipple[17]. The simple ducts are also seen in the dermis of areola at the periphery and seem to extend within 1mm of the germinal layer of epidermis.

In electron microscopy[34] the normal cuboidal to columnar luminal cells within the ducts and ductules show specialized surface microvilli and contains secretary droplets near the apical pole. They rest on the basal lamina or the basal myoepithelial cell layer, which is discontinuous and oriented at right angles to the luminal cells. Their cytoplasm shows mitochondria and the Golgi apparatus.

Myoepithelial cells show contractile actin filaments in the cytoplasm. The varying cytoplasmic densities, the light and dark, of epithelial and myoepithelial cells may probably related to difference in level of differentiation from its precursor cells. Lymphoid cells are also often noted between these two layers.

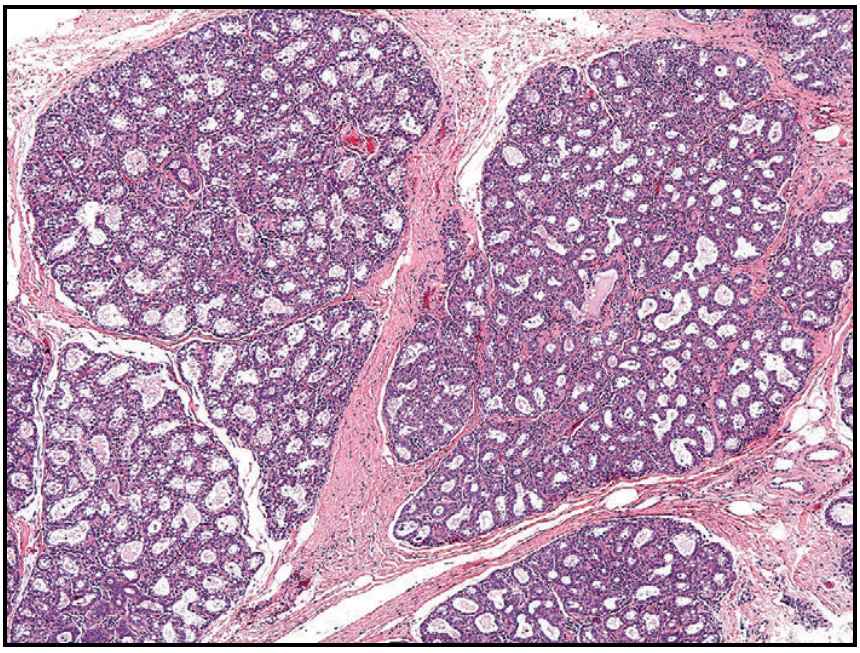
Fig - 8 Electron micrograph of Normal Breast tissue

Asterisk mark shows microvilli on the luminal aspect of epithelia cell. Nucleus is in euchromatic state.

Pregnancy[26, 32]

The complete development and differentiation of the human breast lobular structure takes place only during pregnancy.

Fig - 9 Histology of Breast during Pregnancy



Terminal duct lobular units with luminal secretions and vacuolated cytoplasm seen. Intra lobular stroma is not seen due to expansion of terminal duct (Low power view).

In pregnancy, at the first trimester, the epithelium resumes its proliferation leading to marked increase in the number of lobules and number of acini or ductules per lobule. This is mainly due to epithelial proliferation and lobulo alveolar differentiation by estrogen and progesterone respectively, along with prolactin and growth hormone.

These hormonal effects are enhanced by adrenal hormones, like glucocorticoids and insulin. The lobular development and its expansion takes place at the expense of both intra lobular and inter lobular stromal tissue.

During the second trimester and the third trimester, the lobular growth continues with a mono layered appearance in the acini, as the myoepithelial cells are not readily seen, due to increase in epithelial size and volume. But, the myoepithelial layer cells in the intra or the interlobular ducts are seen clearly. The cytoplasm of the epithelial cells shows vacuolization. The secretions are accumulated in the expanded lumen of the acini. The type of lobule (Russo et al) predominantly seen in pregnancy is Type IV.

Pregnancy related changes

The changes in pregnancy can cause alarm in an in experienced observer, if the person is not aware of it, like areas of infarction widely seen during pregnancy. The prominent nuclear and nucleolar enlargement in the epithelium may be mistaken for atypical ductal or lobular hyperplasia or even for malignant change.

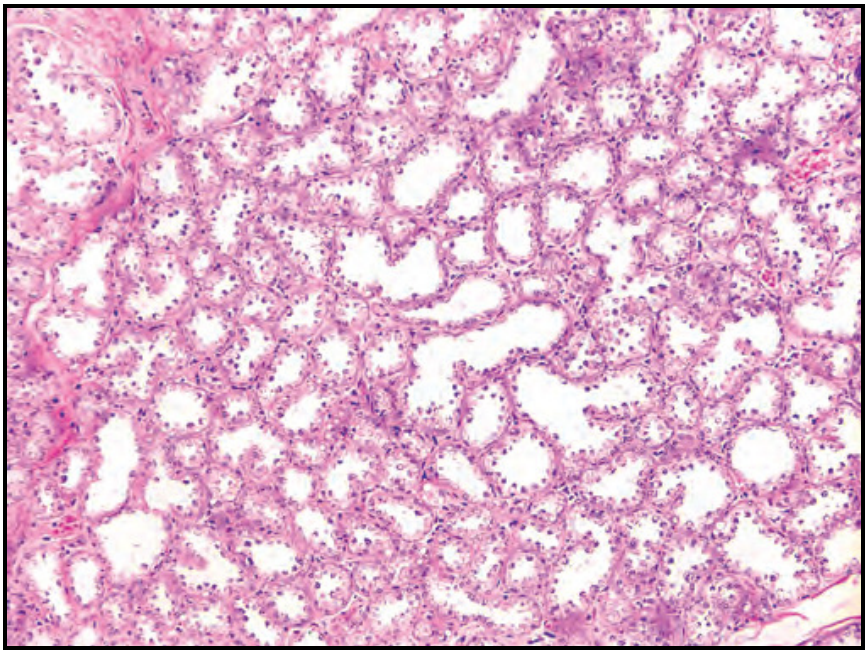
During third trimester, sometimes the increase is the breast parenchymal growth may cause palpable and mammographically detectable masses called lactation adenomas.[41] These are localized collection of lobules seen separately from the rest of the lobules, in pregnancy. The nodules or adenomas are due to slow involution of these lobules as compared to the rest of the lobules in the breast. These nodules are not neoplastic but are variations form normal physiological state of lactating breast.

Lactation

After parturition, the mammary gland shows distention of lobular acini with collection of abundant secretions.

The epithelial cells show prominent cytoplasmic vacuolization, prominent nuclei and nucleoli. They also show hob nail appearance (apical snouting) due to the bulbous protrusion into the lumen. The myoepithelium, as seen in pregnancy, is difficult to discern due to attenuation. The type of lobule (Russo *et al*) predominantly seen lactation is Type IV.

Fig - 10 Histology of Breast during Lactation



There are numerous dilated acini in this lobule with minimal intervening stroma. (Low power view).

Involution[26]

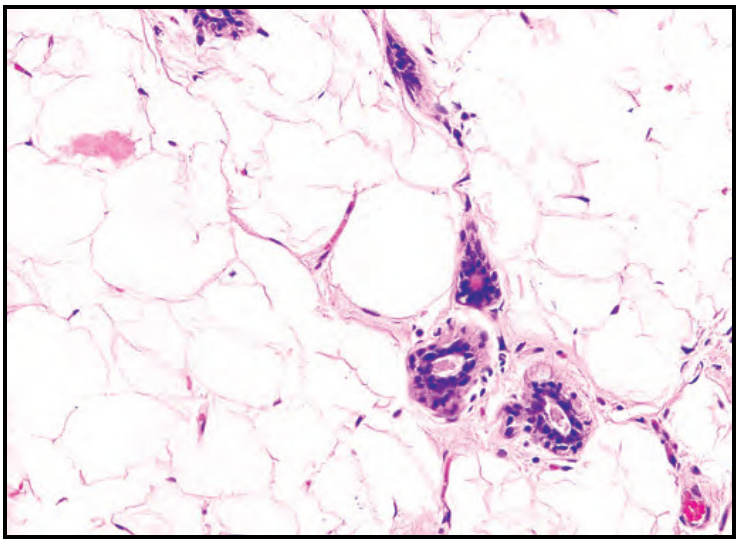
When lactation stops, the lobules of the mammary gland undergoes involution to resume the normal resting histological appearance. This process of involution may take several months. The involuting breast lobules show irregular contour and show infiltration of lymphocytes and plasma cells in increased number in the stroma. The predominant lobular type is type III or IV after involution.

Rarely in non-lactating breast, may isolated lobules may show secretory changes a seen in lactation. This is called Residual Lactating lobule. This may also be seen in nulliparous women.

Menopausal changes[29]

Menopause occurs in old age as a result of atresia of more than 99% of the ovarian follicles (around 4, 00,000) which are present in the ovaries of fetus at a gestation age of 5 months.

Fig - 11 Histology of Breast during Menopause[32]



Postmenopausal breast tissue shows few atrophic ductules in a fatty stroma (High power view).

During the perimenopausal period, the ovulation of lobule is irregular due to insufficient rise in the estrogen level to cause LH surge or the remaining ovarian follicles no longer responds to the hormonal stimulus. The characteristic sign of menopause is amenorrhea, due to complete stoppage of the production of ovarian hormones, estrogen and progesterone.

During the post-menopausal period the decrease in the level of both hormones leads to involution and atrophy of lobulo acinar structure leading to decrease in the size and complex arrangement of acini. The specialized stroma in the intra lobular part of the lobule also decreases. This ultimately results in their replacement by adipose tissue and leads to the presence of only remnants of the Terminal duct lobular units (TDLU). It consists of atrophic acini and absent intra lobular stroma embedded in fatty tissue. The lobular type (Russo et al) is predominantly type I with fewer amounts of Type II and III. After menopause, regression of the breast parenchyma occurs both in nulliparous and parous women.

Developmental abnormalities of breast[34]

1. Complete absence of mammary gland either unilateral or bilateral is very rare.

2. Small hypoplastic breast may be seen in association with Poland syndrome (Pectoral dysplasia – dysdactly. In this syndrome, there is unilateral absence of Pectoralis major and minor muscles; abnormal development of chest wall along with breast, arm and hand of varying degrees may occur.

3. Finlay Marks scalp – ear – nipple syndrome is an autosomal dominant, rare disorders. Nipple and breast hypoplasia or agenesis along with cataract and renal abnormalities are seen.

4. Pallister- Schinzel Ulnar- mammary syndrome is another autosomal dominant condition with limb defects, abnormalities in apocrine and mammary glands. The mutation seen is in the TBX gene, which encodes the T box transcription factor.

5. Mammary gland hypoplasia may also be seen in hypohydrotic or anhydroitic ectodermal dysplasia syndromes.

6. Minor degree of asymmetry is common which is considered normal. Marked degree of asymmetry, which includes small hypoplastic breast with contralateral normal breast development, or juvenile hypertrophy, are more common and may cause severe pain and body image problem in affected young women.

7. Asynchronous thelarche is not uncommon. Any misinterpretation of this as a pathological process may lead to unnecessary and inappropriate surgery . This may severely disrupt the subsequent growth and ultimately may require cosmetic correction. Premature thelarche, if occurs, may regress and only some develop true precocious puberty followed by normal development.

8. Most common variation of mammary gland development is supernumerary nipples with or without breast tissue. Usually seen along the milk line from axilla to groin. They may be enlarged during pregnancy and may secrete milk during lactation.

9. Accessory breast occurs 1-3% of males and twice as much in females. They may be seen in more than one site and are most commonly seen is the thoraco-abdominal region, with left side predominance. These accessary breast tissues may develop any of the breast lesion to which normal breast tissue is susceptible.

10. Polythelia (extra nipples), if occur, they are usually in infra mammary in position.

CHANGES IN THE BREAST IN RELATION TO AGE

The study of morphological changes of human breast tissue in relation to age is important, as age is an important risk factor for developing carcinoma breast. As known, the development of human breast starts in the early embryonic stage and continues well after birth with increased growth noticed during puberty, pregnancy and lactation. The development of breast is a process of simultaneous interaction between the development and differentiation.

Russo and Russo[35] has classified the lobular architecture of breast, using whole mount preparation into four types, which represents the order of each stage of development, postnatally. The identification of the histomorphology of each type and quantification of the lobule is essential part of the evaluating the effect of age and parity on the human breast tissue.

Russo and Russo (1992)[29] based on the comparative study in rat and human mammary gland, stated that the degree of differentiation of the breast determine the degree of protection afforded to the breast tissue from carcinogenesis during the reproductive period [9]. In the rodent studies the initiation of carcinogenesis is inversely proportional to the degree of differentiation, which again depends on the age and the history of reproduction.[8, 43]

In the comparative studies between human and rat mammary glands to understand the pathogenesis, Russo *et al*[42] proposed that the site of origin of carcinoma in human breast is the Terminal duct Lobular unit (TDLU). TDLU is also the site of initiation preneoplastic conditions like, atypical ductal hyperplasia (ADH), which progresses to in situ carcinoma of ducts, later evolving is an invasive ductal carcinoma.

Russo *et al*[42] in his study stated that

1.The terminal duct lobular unit is equivalent to Lobular type I of Russo's classification.

2.Lobular type II might evolve into ADH and insitu carcinomas.

3.Lobular Type III might evolve into other benign lesions like fibroadenoma, benign apocrine cystic lesions and hypersecretary lobules, and scerosing adenosis.

As the lobular types are classified by Russo based on degree of differentiation, his observation suggests that the tumor development, both benign and malignant is based on the degree of differentiation.

Dawson *et al*[16] in his study also underlined the important of the knowledge of architecture of the normal human breast and the influence on of its development on the potential of the organ to develop malignancies.

Wellings *et al*[17] also has emphasized the findings of Dawson *et al*. Several other authors, Geschickter *et al,*[81] Ingleby *et al,*[44] also agreed with the findings of Dawson *et al* and Wellings *et al*.

CHANGES IN THE BREAST IN RELATION TO PARITY

The development of breast cancer in human breast tissue is greatly influenced by the reproductive history of the women,[35] Full term pregnancy gives maximum protective effect on the breast tissue as compared to the nulliparous women and in women who did not complete the pregnancy.

In the study by Russo and Russo[36] using whole mount tissues, 51 samples obtained from bilateral and unilateral reduction mammoplasties including samples from parous and nulliparous women, he stated that,

1. The terminal ductal structures defined as straight blind ending or club shaped terminal ducts with no connection with lobular structures is seen in 10% of tissues from age group between 14-18 years.

2. Decreases to <1% after that up to the age of 40 years.

3. There after the value increases significantly.

According to his study, Russo *et al* reports,

1. Lobular type I is present is equal proportion to lobular type II and III between ages 14-18 years. From 23-40 years

Lobular type I decreases in value; after 40 years the value increases to become the predominant type (70%) of the total lobules, and thereafter.

2. Lobular type II in the age of 14-23 years is equivalent to that of lobular type I and III. After 23 years, up to 40 years, there was no significant increase in the lobular type II, and remains unchanged (20%). After 40 years it decreases and by fifth decade, the value is around 5%.

3. Lobular type III is present in equal proportion with type I and II during the age group 14-23 years. After 23 years, the value of lobular type III increases, and becomes the predominant type (70%) by the third decade and remains so up to fourth decade. After forty years, Lobular type III decreases significantly and the value in fifth to sixth decade is around 15%.

Russo *et al* therefore proposed that the mammary gland lobular architecture shows positive correlation between age and Terminal End Bud (TEB) / Lobular type I, negative correlation between age and Lobular type III. Lobular Type II does not show any correlation with age. Suggesting involution of Lobular type III to lobular type I with age.

On the comparison of the lobular architecture with the reproductive history, i.e between nulliparous and parous women, Russo *et al* stated that significant differences were seen in the two groups.

In nulliparous women,

1. All the age groups showed predominance of Lobular type I (50%) with slight increase after 20years up to the third decade. Thereafter remains constant remains constant 6th decade.

2. The second most common type is lobular type II (20-30%) up to 23 years and decreases thereafter. Terminal duct structure was approximately 10% and remains constant up to 4th decade. After 40 years slight increase 20%) was seen.

3. Lobular type II was rarely seen in nulliparous breast tissue of all ages (<10%).

In parous women,

1. Most common type is lobular type III. Between 14-18 years showed 100% value. After 2nd decade, the value of the lobular type III was 70-80% up to fourth decade. After fourth decade, the value

drastically decreased up to 20% and by sixth decade of life, it decreased up to 10%.

2.Lobular type II also shows similar decrease between 2nd and 4th decade with value of 25%.

3. Lobular type I increase greatly 4th decade.

The above findings suggest that, the involution of lobular type III to type I becomes similar between similar between parous and nulliparous women after the age of 40years. It also suggests that in nulliparous breast tissue, the predominant lobular type is Type I and is constant in all ages. This represents that in nulliparous women, the degree of maximum differentiation is never reached as seen in parous women, due to the absence of the effects of pregnancy.

In another study by Russo *et a,l*[35] the changes in the breast between nulliparous and parous women were recorded in women having malignancy with that of non-malignant cases. In this study, group I nulliparous women without carcinoma, and group II, nulliparous women with carcinoma, showed no significant change in the lobular type. Both group I and group II showed lobular type I as the predominant type.

Lobular type II as less common and type III as least common types. The difference in percentage of lobular type I and III showed great significance but not between the two groups.

Group III, parous women without cancer, showed lobular type II and III as the predominant type, as compared to lobular type I (25%). Group IV, parous women with cancer showed very high proportion of lobular type I (>50%) with <25% of lobules showing lobular type II and III. The above results in the study suggest that the predominance of lobular type I in parous women with cancer (group IV) is similar to that of nulliparous women (both group I and II). And therefore, implies that the degree of differentiation in the breast tissue of parous women with cancer is not complete as expected after pregnancy.

The result confirms the observation of other studies that lobular type III with higher degree of differentiation affords more protection from breast cancer. Hence, Russo *et al* suggested that the architectural pattern of the breast could represent significant end points, which can be used as a measured of response in the mammary gland, after chemopreventive therapy.

As pregnancy induced differentiation in the breast tissue is mainly due to estrogen and progesterone hormones, studies show controversies in the effect of these hormones on the cell proliferation (Ma Carty, 1989), [45] (King 1990).[46] The controversies were reflected in the data, from tissue culture samples and xenografts of nude mice and from the assumption of effect of these steroidal hormones on both endometrium and normal breast tissues.

Even though, studies on the receptor status of endometrium (both estrogen and progesterone) were clear, (Lessey et al 1988), [47] the same is not true about the steroid receptor status with menstrual cycle and its consistency.[48, 49]

Battersby *et al*[39]conducted immunohistochemical study of estrogen (OR) and progesterone (PR) receptors present in the histologically normal breast epithelium in relation to age, parity, menstrual cycle and the effect of oral contraceptive usage. He studied the effect of age on the OR and PR receptor using both chronological age and breast age, time since menarche. The results showed no significant changes in both the OR and PR receptor status. He noted that the parous women in natural menstrual

cycle, without OCP use, time interval since birth showed effect on the OR Positive cases and its scores.

The reactivity was more frequent after 5 years of last child birth as compared to < 5 years duration. PR reactivity also showed decreased value among women who had child birth in <5 years as compared with nulliparous women. The explanation for these changes was proposed to be based on histological observation of marked atrophy of the terminal duct lobular unit (TDLU) leading to local refractory effect of steroid receptors to endocrine factors. An understanding of the mechanism behind this refractory effect can be useful in preventing or reducing the risk of carcinoma breast.

Morphometric studies on age related change in the normal human breast tissue was done by Hudson *et a.l*[50] He studied the effect of these changes in the evolution of mammary carcinoma. In his study, sub gross and microscopical analysis of sub cutaneously removed mammary glands from female subjects of autopsy was done. The sample size was 58 breast form 10-80 years divided into groups of eight.

On examination of the tissues at sub gross level both qualitatively and quantitatively, the findings of the study showed maximum number of lobular units, appeared earlier in the upper quadrants than lower quadrants. The upper outer quadrant showed relatively large volume of lobular units, except for the fifth decade. The level of fibrous tissue decreased between 3rd to 6th decade, and the level of fatty tissue increased.

The results were reversed in the 7th decade of life. The epithelial tissue volume, both acinar and intralobular ducts increased. Between 2nd to 5th decade and decrease during 6th decade. The volume of acini and intralobular duct lumen was constant except in 3rd decade, showed decrease in value. The volume proportion of blood remained constant.

With increasing age, the breast morphometry showed decrease in the mean diameter and volume assuming the lobule as spherical in shape, There was no correlation between the number of epithelial tissue and pregnancy in any of the breast tissue of different age groups.

The maximum value of epithelial tissue volume was found in 3rd decade, declines during post menstrual phase.

From the above observations, Hutson *et al* concluded that, the epithelium in all the quadrants of breast varied with age increasing during 3rd decade and declining thereafter. Histologically, the volume of lobular units including both epithelial and connective tissue components, was more in the upper outer guardant which correlates with higher incidence of carcinomas in this quadrant.

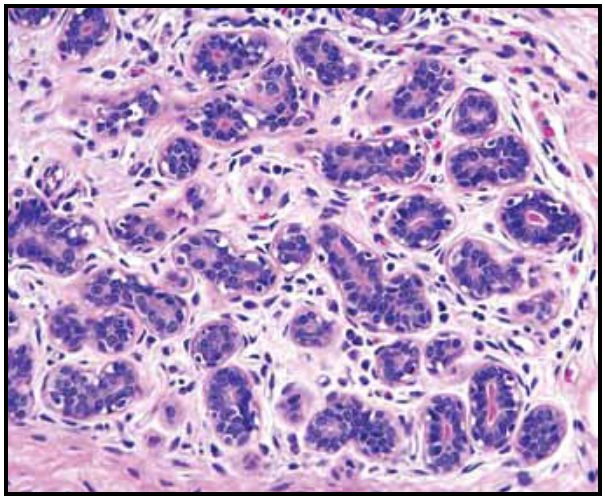
No significant changes were found in the size of the lobule between the four quadrants. But size decreased in relation to advancing age. Epithelial tissue and fibrous tissue volume also decreased with age.

CHANGES IN THE BREAST DURING MENSTRUAL CYCLE

Lobules under the influence of hormones secreted by the ovary, exhibits changes in the epithelial and stromal aspects. The changes vary even from the adjacent lobules and among different lobules in the same breast, but a dominant pattern exist in each phase of menstrual cycle. But the changes due to the hormone action are very subtle in cases of menstrual cycle as compared with that of changes during pregnancy and lactation.

Follicular phase[32]

Fig - 12 Histology of Breast in Follicular phase



Myoepithelial layer not conspicuous. Luminal cells show round nuclei, lightly stained cytoplasm and eosinophilic intra luminal secretions. Rare vacuolation is seen. Intra lobular stroma shows infiltrate. Mitosis absent.

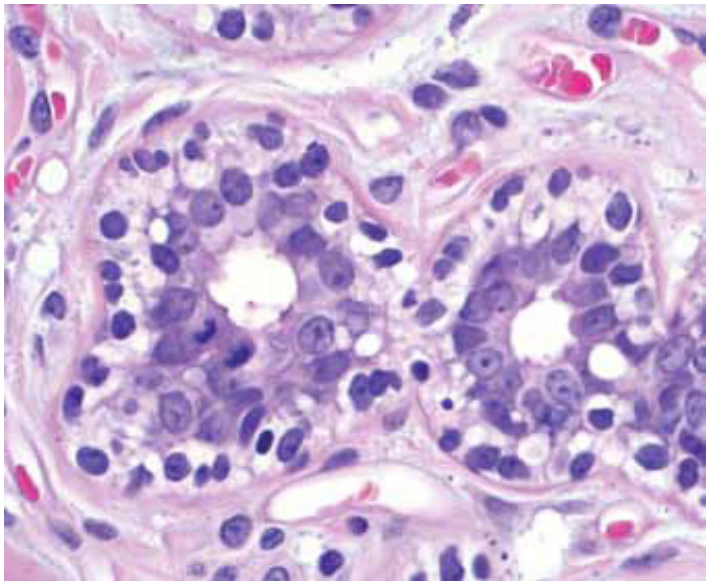
Table - 2 Morphological criteria for studying changes in breast during phases of menstrual cycle[32]

Phase of Menstrual cycle	Epithelium	Morphology of lobule	Intra lobular stroma
I (Early follicular)	Bilayered polygonal cells with inconspicuous myoepithelial cell layer. Mitosis- rare.	Lobules are small with few acini showing closed lumen. No secretions are seen.	More cellular and dense.
II (Late follicular)	Three layered. Myoepithelial cell layer is conspicuous. Mitosis –rare.		Less cellular. Collagen bundles are more.
III (Early luteal)	Hob-nail appearance of the luminal cells (apical snouting). Myoepithelial cell show vacuoles. Mitosis - rare.	Lobular size and number are increased. Lumen is distended with secretion.	Lymphocytic infiltration seen. Stroma is edematous and loose with congested blood vessels.
IV (Late luteal)	Hob-nail appearance of the luminal cells seen. Myoepithelial cell show vacuoles. Mitosis seen	Size of the lobule and the acini are very much increased. Lumen is distended with abundant eosinophilic secretion.	
V (Menstrual)	Few of the luminal cells show hob nailing. Myoepithelial cell show vacuoles. Mitosis - rare.	Degenerating epithelium and reabsorption of secretions seen	Cellular and dense.

In this half of menstrual cycle, the intra lobular stroma is dense and fibrous with increased cellularity. The epithelial component shows luminal cell layer with orientation around a tight, closed lumen. Myoepithelial cells are inconspicuous during this phase. Mitotic figures are absent or very rare.

Luteal phase

Fig - 13 Histology of Breast in Luteal phase of menstrual cycle[32]



In the above picture, the epithelial cells show prominent nuclei and nucleoli with basophilic cytoplasm. Vacuolation; few mitotic figures and apoptosis; stromal edema with increased inflammatory infiltrate are also seen (40x).

In the second half of the menstrual cycle, the intra lobular stroma is loose, myxoid and edematous. The epithelial cells lining the lumen show prominent nuclei and nucleoli and basophilic cytoplasm. The lumen is distended and filled with eosinophilic secretions myoepithelial cells are evident and their cytoplasm shows vacuolation with centrally placed or apical nucleus. Mitotic figures may be seen during this phase.

The interlobular stroma is relatively unaffected in both half of the cycle. Occasionally, the epithelial cells may show clear cell cytoplasmic changes both in the premenopausal and post-menopausal women not related to pregnancy or exogenous hormone use.

Rosenberg (1922)[51] was first to note changes in the histology of mammary gland in relation to menstrual cycle. He described the cyclical event of proliferative premenstrual lobulo-alveolar proliferative changes followed by regression and atrophy of glandular tissue during post menstrual period , based on autopsy material.

Deikman (1925)[52] and Moskowitz (1926)[53] in their studies on the changes in connective tissue elements during the menstrual cycle and considered epithelial changes as secondary changes. They contradicted

Rosenberg's observation of post-menopausal regression. Also they emphasized the premenstrual change in the gland, the lobulo alveolar dilatation, as "true apocrine section".

Centeno (1927)[54] and Palano (1924)[55] compared the lobulo alveolar proliferation of breast tissue during premenstrual period to that of pregnant breast in first trimester, which supported Rosenberg's observation.

Speert (1941)[56] in his study to resolve the problems of the earlier studies due to use of human breast tissue studied the changes in rhesus monkey. He proposed the lobular enlargement in premenstrual period as secondary change to the dilatation of acini and stromal edema.

Geschicter (1945) [18] in autopsy and surgical materials described two different phases in the breast tissue.

Phase I – (regression phase: day 1-8) showed epithelial atrophy and closed lumen in the acini, condensation of the intra lobular stroma with variable degree of inflammatory cell infiltration.

Phase II – (Proliferation phase) showed proliferative changes with lobulo-alveolar budding, secretions in the lumen of the acini and edema in the intralobular stroma.

Ozzello *et al* (1958)[57] in his study demonstrated increased content in the intralobular stroma during the premenstrual state of menstrual cycle which may account for altered connective tissue.

Zeppa *et al*(1969)[58] in his study noted that the intralobular stromal edema and venous congestion leading to distortion and separation of terminal lobular units, as a result of histamine like effect of sex steroids hormones on the mammary gland micro-circulation during premenstrual phase.

Bassler *et al* (1969)[59] in his ultra-structural study on human breast, tissue noted the response to exogenous estrogen as epithelial differentiation. He described 2 types of cells "A" and "B" cells. The cytoplasmic filaments of "B" cells (chief cells) resembled the precursor cell for both luminal and myoepithelial cells.

McMohan *et al* (1970)[60] proposed the early pregnancy decreases the risk of breast cancer.

Haagensen (1971)[61] in his semi quantitative study did not find statistically significant difference for any of the specific morphological components in relations to menstrual cycle.

Randnor (1972)[62] in his studies noted the differentiation of epithelial tissue in response to the hormonal level changes. He also suggested that the presence of progenitor cell gives rise to both lineage of cells in luminal and basal epithelial cells.

Fanger (1974) [63]in his ultra-structural study of menstruation dependent cyto differentiation in the breast tissue of humans noted increased basophilia in the luminal epithelial cytoplasm due to increased RNA and cytoplasmic content. He also noted increased rough endoplasmic reticulum and vacuoles containing secretions during premenstrual (21-27) days, including active protein synthesis and secretion.

He also proposed that this morphological expression of increased transcription and translation was due to the action of estrogen hormone stimulation on the cells. He also noted increased chromasia, nuclear enlargement and basal migration in the luminal cell. According to his study, both luminal and basal layer resembled the "A" and "B" cells of Bassler.

Meyer (1977)[64] noticed high mitotic index in the second half of the menstrual cycle using TLI (Thymidine Labeling Index) and apoptosis peak at 28th day of the cycle.

Vogel *et al* (1981)[10] in his study on histological changes in the breast during menstrual cycle described a histological criterion which consists of reproducible morphological categories in five phases based on chronological age of menstrual cycle. He stated that, the space specific changes in the breast are seen in both stromal and epithelial components.

His studies contradicted the observations made in subsequent studies, in that the proliferative changes were observed during menstrual period. They include, compact lobular and acini with frequent mitosis from day 3 to7.He sites documentary support from animal experiments done using increasing serum level. He noted the proliferative activity as irregular stratification and apical budding in the epithelium.

Ferguson and Anderson *et al* (1981a) [13] noted the significant cyclical variation in apoptotic activity that were evident in their study with mathematically adjusted values for both mitotic and apoptotic frequencies with that of day of menstrual cycle.

Pike *et al* (1983)[65] noted increased incidence of breast carcinoma after prolonged exposure to high dose of exogenous progesterone, as in oral contraceptive pill users.

McManus and Welsch (1984) [66]in their in vitro studies using nude mice, showed the stimulant action of estrogen on proliferation of normal breast epithelium.

La Vecchia *et al* (1985)[67] stated that regular menstrual cycles are likely ovular in nature and so corpus luteum secretes progesterone during these cycles.

Longacre and Bartow (1986)[11] in their comparative study of endometrium and breast epithelial lining during the menstrual cycle observed high mitotic activity in the latter half of the cycle.

Murphy *et al* (1986 a, b)[68] proposed that progesterone under experimental conditions causes stimulation, as indicated by its mitogenic action in vivo in women. It also primes the epithelium to respond to growth promoting peptides as proved by its stimulation of lactogenic and epidermal growth factor receptors in tumor cells of mammary gland.

Going *et al* (1988)[12] in his study of both proliferative and secretory activities of human breast, both during natural and artificial menstrual cycles, found increased proliferation during second half of the cycle. He also found no significant correlation or relation between proliferatory and secretory activities of the resting breast tissue as seen in endometrial tissue.

Ferguson (1988)[69] in his ultra-structural study of mitosis and cell kinetics of normal breast tissues in human suggested that luminal cell gives raise to both the luminal and basal epithelial cells.

Potten *et al* (1988)[70] in his study of cell kinetics, used autoradiography and proliferative indices like number of epithelial cells/lobule; Labeling index (LI), after treating the tissue with tritiated Thymidine (3HTdR); Mitotic index (MI) and apoptotic index (AI). He proposed, based on the above criteria, the proliferative activity of normal breast of humans' declines with age and the maximal activity was seen during the second half of menstrual cycle. He also propose an association between progesterone and increase risk of carcinoma, due to increased proliferative index found in the latter half of menstrual cycle during which progesterone levels are high.

Batterby and Anderson (1989) [71]stated that histology of TDLU post pregnancy ($\leq$ 5 years) show marked atrophic changes and may readily account for its refractory behavior to local environmental factors.

Jacquemier *et al* (1990)[72] stated that progesterone receptor level increased during second half of menstrual cycle and therefore show cyclical variation with cycle.

Joyenx *et al* (1990)[73] proposed increased localization of fatty acid synthase (a marker for progesterone receptor) during the 2nd half of menstrual cycle in the breast tissue.

Williams *et al* (1991)[74] in his found no cyclical variation in the progesterone positivity with the menstrual cycle.

Batterby *et al* (1992)[75] in their semi quantitative analysis of OR and PR reactivity in epithelial nuclei of normal breast tissue proposed that there is heterogenic reactivity of immune reactive receptors within and between the TDLU. Also, noted that OR level significantly increased during the first half of cycle and decreased during the first half of cycle and decreased during the second half of cycle, probably due to down regulation by progesterone action and the PR reactivity showed no cyclical variation with menstrual cycle.

He also stated that time since pregnancy showed major effect on the steroid receptor expression, as evidence of altered tissue environment following pregnancy as suggested by Anderson et al (1989)[76].

Andres and Strange (1999)[77]stated that proliferation or apoptosis were isolated and distributed in various lobules and are present close to the lumen. The apoptotic activity did not vary significantly in between the two phases in contrast to the study by Ferguson and Anderson (1981a).

Rathi Ramakrishna *et al* (2002)[78] in their study found increased mitotic count in the lobules of breast tissue during the second half of cycle (luteal phase) and the proliferative activity was confined to the luminal epithelium.

They also noted correlation between the morphological stage and chronological phase in the normal lobules of the surgical specimen. They have evaluated the histological changes of breast tissue with the phases of menstrual cycle using the following features.

Epithelial – Myoepithelial distinction

(i) Percentage of myoepithelial cell showing vacuolation

(ii) Degree of vacuolation in myoepithelial cell

(iii) Luminal sharpness in the acini

(iv) Presence of luminal secretion

(v) Stromal edema and infiltrate

(vi) Mitotic activity in the luminal cells

The lobules were scored using 0-3 levels and summarized the correlative response into 4 stages.

Stage I (Early Follicular) Morphological score: 0-5

Day of the cycle: 0-5

Stage II (Late Follicular) Morphological score: 6-9

Day of the cycle: 6-15

Stage III (Early Luteal) Morphological score: 10-15

Day of the cycle: 16-24

Stage II (Late Luteal) Morphological score: 16-19

Day of the cycle: 25-28

Rathi Ramkrishnan et *al* (2004)[79] observed no association between the lobular architecture and breast cancer risk. They also found association of lobular type with menstrual phase using the morphological criteria but no association was noted when date of the cycle was used for correlation.

Mari Alicia H Navarrete *et al* (2005)[79] studied the Proliferative index (PI) [using Ki 67] and noted that the index higher in the luteal phase than in follicular phase. The apoptotic index (AI) [using TUNEL positive cells / 1000 epithelial cells] showed no statistically significance between the two phases of menstrual cycle. The cell renewal index (CRI) i.e. PI/AI showed significant increase in luteal phase. PI also positively correlated with the serum progesterone levels as seen in animal models.

MYOEPITHELIAL CELLS

Myoepithelial cells are localized between luminal epithelial cells and the stroma, an ideally position for them to communicate with both components. Recent studies indicate that myoepithelial cells may function as a guardian of tissue integrity in the human breast by maintaining tissue polarity[80, 81].The loss in polarity is consistent with the hypothesis that fully differentiated myoepithelial cells are natural tumor suppressors. Both *in vitro* and *in vivo* studies have confirmed the ability of myoepithelial cells to suppress tumor growth and invasion[82, 83].

The hallmarks of breast cancer progression are the loss of normal tissue architecture, including polarity. It is also postulated and generally accepted that primary breast carcinomas show a marked increase in the ratio of luminal-to-myoepithelial cells, and that many invasive breast carcinomas essentially lack myoepithelial cells entirely[84]. Normal myoepithelial cells are critical for correct polarity of luminal epithelial cells, most likely via production of laminin-1.

Recent data states that it is also possible that the tumor suppressive ability of myoepithelial cells depends on their complete differentiation and that changes in their expression pattern can lead to reversal of their function, i.e., that undifferentiated myoepithelial cells may actually promote tumor progression instead of suppressing it. It is interesting to note that this is the classical pattern of tumor suppressor molecules, such as p53, and once organ integrity is breached, epithelial cells may alter their signaling molecules to act like oncogenes.

The role of myoepithelial cells as possible tumor suppressors may include their function as a 'guardian of normalcy', being a paracrine inhibitor of invasion in early breast cancer as well as a target for differentiation therapy by inducing malignant cancer cells to differentiate along the myoepithelial pathway to a less devastating cell type.

Changes to the balance of Human Mammary Epithelial Cell (HMEC) lineages in the epithelium may presage susceptibility to breast cancer. Functional analysis of the aging process in humans showed that lobules involute with advancing age leaving behind ducts and residual lobules with changed morphology [85, 86]

The representation of luminal epithelial, myoepithelial, and progenitors may change with age. The hypothesis is that the age dependent changes arose either through age dependent shift in the proportions of luminal epithelial and myoepithelial cells used to establish the strains and/or through intrinsic changes to functional properties of luminal epithelial or myoepithelial that would aid survival or propagation of one lineage. These are evidence of age dependent functional changes in luminal epithelial that could alter their ability to bind extracellular matrix (ECM) and survive in culture.

Known age-related changes include increased adipose, decreased connective tissue[85, 87], decreased overall density[88], disruptions in the basement membrane[89], and changes in protease expression[90]. These types of changes may alter distributions of the different epithelial lineages because human mammary progenitor cell fate decisions can be influenced by changes in microenvironment.

Immunohistochemistry (IHC) method for fixed and paraffin embedded human breast biopsies were reported for detection of myoepithelial cells using antibodies to myosin smooth muscle actin, S100 and p53.Myoepithelial layer is present around normal ducts and lobules. Sometimes they are difficult to identify in H & E stains. Using the IHC markers they are clearly distinguished in the normal breast and benign diseases of breast.

MATERIAL AND METHODS

Source of Data

Normal breast tissue from the patients undergoing breast surgery for benign breast disease like fibroadenoma; breast abscess; and from the normal breast tissues taken from mastectomy specimens, for whom surgery was done for both benign and malignant lesions.

Inclusion Criteria

- All consenting female patients between 11 to 60 years of age.
- All consenting women in good health in the reproductive age group (15 to 45 years) with regular menstrual cycle and known last menstrual period; and no history of intake oral contraceptive pills for the past 6 months from the date of surgery.

Exclusion Criteria

- Age less than 10 years and more than 60 years.
- Women with irregular menstrual cycle in the reproductive age group.

- Women with history of intake oral contraceptive pills for the past 6 months from the date of surgery.
- Pregnant and lactating women.
- Breast tissue from male patients.
- Samples from core needle and true cut biopsies.

Collection of data

Oral informed consent was taken from the patients about the purpose and method of collection of samples. A detailed history of the Age; Parity; Regularity of menstrual cycle; history of intake of oral contraceptive pills for the past 6 months from the date of surgery and history of any debilitating systemic disorders taken.

The sample from benign breast diseases was taken 1 cm away from the surgical margins as they are usually well circumscribed lesions. In the mastectomy specimens, 1x1 cm normal tissue was taken 2cm from the grossly tumor free margin.

Method of tissue processing

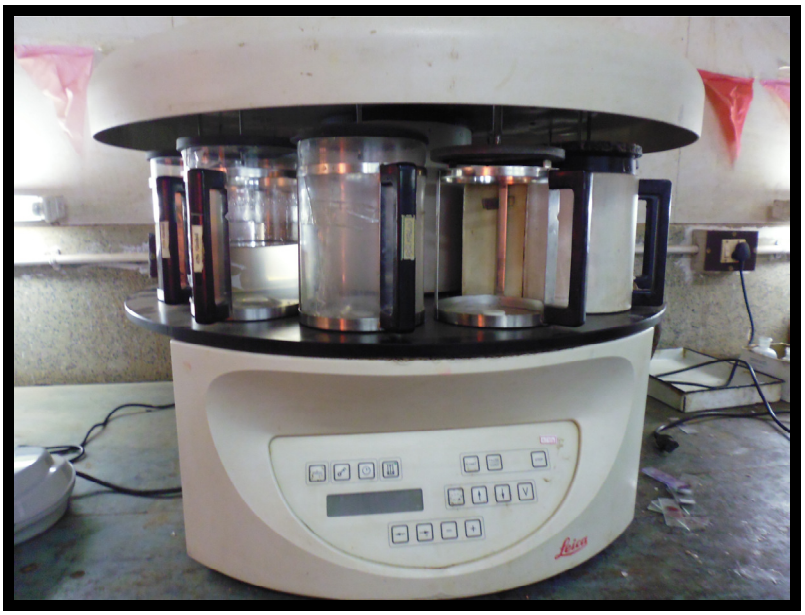
The samples collected were immediately fixed in 10 % Formalin solution, overnight. The fixed tissue was then processed using Automated Tissue Processor (Leica TP 1020).

Procedure

1. Dehydration - with Isopropyl alcohol in ascending grade.

40% - single change 30 minutes; 50% - single change 30 minutes; 70% - single change 2 hours; 90% - single change 6 hours; Absolute alcohol – two changes 2 hours each.

Fig - 14 Automated Tissue Processor (Leica TP 1020)



2. Clearing - done with Xylene (Sulphur free).

First change - 1 hour 15 minutes; second change - 2 hours.

3. Impregnation - done with Paraffin Wax (Melting point 55°- 60° C) First change and Second change - 2 hours each.

The impregnated tissue was then embedded in molten Paraffin Wax (Melting point - 55° to 60° C) using Leuckhart's L molds and the block was cooled and labeled. The blocks are then cut into thin section of 3-4µ in thickness using Rotary Microtome (MT – 1090A) and floated in Tissue Flotation Bath (Dalal) and mounted on the glass slide coated with Meyer's egg albumin.

Rotary Microtome (MT – 1090A)

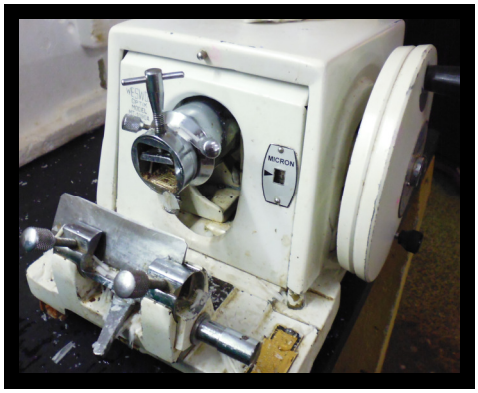


Fig - 15

Tissue Flotation Bath (Dalal)

Fig - 16

The mounted slides were deparaffinized, dipped in Xylene and treated with descending grades of isopropyl alcohol and brought to water.

Staining procedure

After the slide was brought to water, they were stained with Hematoxylin and Eosin stains. Slides were kept in Hematoxylin (Erhlic's) trough for 20 minutes and washed in water. Slides were kept for blueing in running tap water for 30 minutes, then dipped in 1% acid alcohol for differentiation and immediately washed in water. Next slides were dipped for 5 seconds, washed with water then air dried, and mounted using DPX mountant. The slides were then studied under 4X, 10X, 40X magnification, using binocular light microscope (Magnus) and observation noted and analyzed.

Selection of slide

All the slides were screened to rule out any lesion in the sections, both benign and malignant and excluded from the study.

Method used to study age and parity related changes in normal breast tissue

The each screened slide from a total of 36 samples was included and all the normal lobules present (2 to 5) were studied. From each lobule, the number of cut section of ductules was counted and the lobule was typed based on the study of Russo *et al* [29, 35](Table -3) given below.

Table - 3 Lobular Typing of breast tissue (Russo *et al*[29, 35])

Lobular Type #	No: of Ductules /Lobule
I	6 – 11 or TEB*
II	≥ 47
III	≥ 80

Lobular Type IV is seen only pregnant and lactating women.NO sample was collected from these women.*TEB – Terminal End Bud.

Method used to study menstrual cycle related changes in normal breast tissue

Out of the total 36 samples, 11 samples from women with regular cycles, known LMP and negative history for OC pills were included in this part of study. The cycle length of each sample was standardized to 28 days using the following formula (with the assumed length of luteal phase of 14 days).

$$\text{Adjusted day of cycle} = \frac{\text{Length of luteal phase (14)} \times \text{Day of cycle on the day of surgery}}{\text{Length of follicular phase}}$$

The slides were studied mainly in 40X magnification and the observation were noted and analyzed using the morphological criteria of Vogel *et al* with slight modification (Table - 4).

Table - 4 Modified Morphological criteria of Vogel et *al*[10]

Phase	Intra lobular stroma	Acinar lumen and luminal secretion	Epithelium stratification /ME	Myoepithelium vacuolation	Mitosis
I (3-7 days)	Dense, cellular	closed, no secretion	Single layer, no ME	No vacuolation	4/10 hpv
II (8-14 days)	Dense, cellular	closed, no secretion	Triple layer, ME +	No vacuolation	Rare
III (15-20 days)	Loose, edematous	Open, some secretion	ME conspicuous	Vacuolation <30 %	Absent
IV (21-27 days)	Loose, edematous	Open, active secretion	ME conspicuous	Vacuolation >70%	Absent
V (28-2 days)	Dense, cellular	Distended with some secretion	Double layer, ME+	Residual vacuolation	Absent

Immunohistochemistry (S100) - Myoepithelium

To demonstrate the myoepithelial character ,total of 4 slides, one slide each from post pubertal,2 from premenopausal and postmenopausal age each were taken.They all had representation lobular types I, II and III.(Sample nos: 9, 16, 27, 32)

Procedure

Sections were cut and mounted on APES(Amino Propyl triEthoxy Silane) coated slides and treated with 3 changes of acetone (1^{st} and 3^{rd} change for2 mins; 2^{nd} with10ml of APESfor 30 sec).The slides were then dewaxed. Retrival of antigen was done with citrate buffer and then treated sucessively with commercially available Power block, S100 antibody,Super enhancer,SS label and DAB(DiAminoBenzidine) chromogen, after washing with buffer in each stage.Harris hematoxylin was then used as counter stain (1 min), washed dried and mounted with DPX.

OBSERVATIONS AND RESULT

CHANGES IN NORMAL BREAST TISSUE WITH AGE

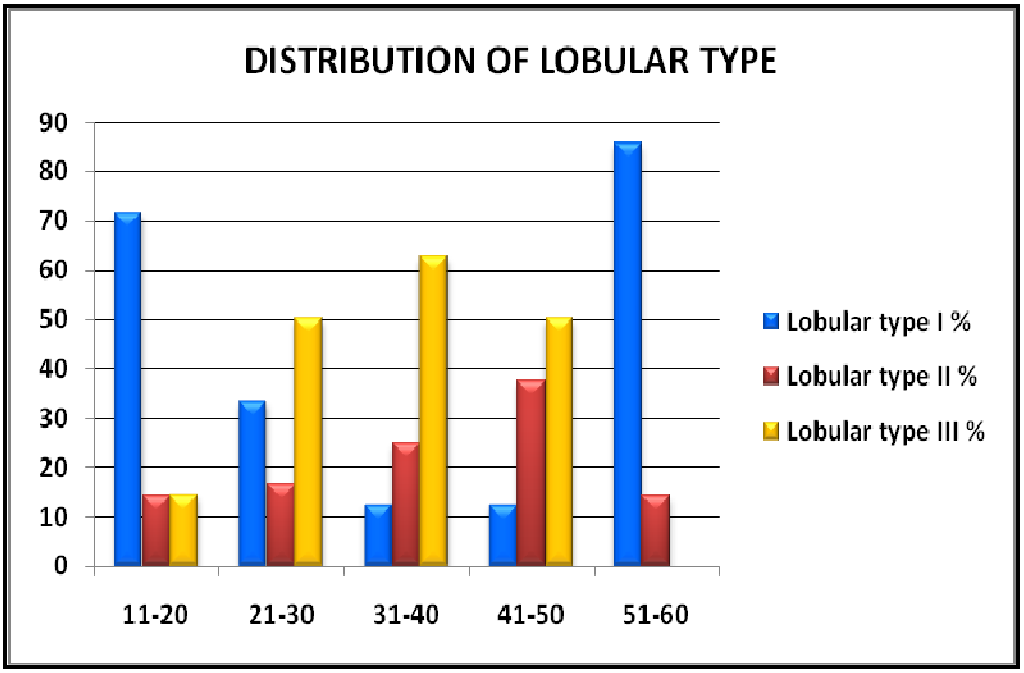
A total of 36 samples of normal breast tissue from surgical patients was done. All sample analyzed were plotted against age group. The ages varied from 15 to 60 years and are divided into 5 groups separated by 10 years interval.

The relationship between age and percentage of lobular structure in the breast samples were noted. The percentage of the lobular structure was identified using the parameter described by Russo *et al* [36] in whole mount preparation. The results were plotted for total population of patient without considering the parity status of the patient.

Table - 5 Lobular Type distributions in different age groups

Age group	Number of samples	Lobular Type I		Lobular Type II		Lobular Type III	
		Number	%	Number	%	Number	%
11-20	7	5	71.4%	1	14.3%	1	14.3%
21-30	6	2	33.3%	1	16.7%	3	50.0%
31-40	8	1	12.5%	2	25.0%	5	62.5%
41-50	8	1	12.5%	3	37.5%	4	50.0%
51-60	7	6	85.7%	1	14.3%	0	0.0%

Chart - 1



The predominance of lobular architecture was determined based on the percentage of the lobules showing a particular type i.e. >50% of the lobule showing a particular type, was considered predominant type in that age group. All the lobules present in a particular sample, varying from two to five in numbers, were taken for typing of lobule. The predominant type of lobule in each age group in given in (Table -6)

Table - 6 Predominant Lobular Type in different age group

Age group	Number of samples	Lobule Type	Number	%
11-20	7	I	5	71.4%
21-30	6	III	3	50.0%
31-40	8	III	5	62.5%
41-50	8	III	4	50.0%
51-60	7	I	6	85.7%

In the age group of 11-21 years, 7 samples were included out of which 5 showed the Lobular Type I (71.4%)of this group). Lobular type II and III were seen in one sample each (14.3% each)

In the age group of 21-30 years, a total of 6 samples were included, out of which three samples showed lobular Type III (50%). The next common type was Type II, seen in 2 samples (33.33%). Lobular Type I was found in one case (16.7%).

In the age group of 31-40 years, a total of 8 cases were included, out of which five samples showed Lobular Type III (62.5%). The next common type was Lobular Type II (25%) followed by Type I (12.5%).

In the age group of 41-50 years ,8 samples were included, out of which 4 samples showed Lobular Type III (50%), followed by Type II (37.5%) in 3 samples. Lobular Type I was seen in one sample (12.5%).

In the age group of 51-60 years, a total of 7 samples were studied out of which 6 samples (85.7%) showed Lobular Type I. Type II was found in one sample (14.2%). None of the samples showed Lobular Type III.

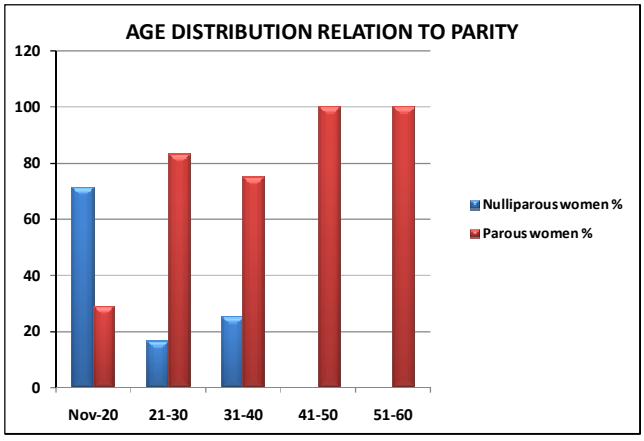
CHANGES IN NORMAL BREAST TISSUE IN RELATION TO PARITY[36]

All of 36 normal breast tissue samples used for the analysis of age related changes were included to study the relation of normal breast tissue to parity using the same age distribution pattern.

Table - 7 Distribution of samples in relation to parity

Age group (years)	11-20	21-30	31-40	41-50	51-60
Nulliparous	5	1	2	0	0
Parous	2	5	6	8	7
Total	7	6	8	8	7

Chart - 2



The distribution pattern of lobular types among nulliparous and parous women in each age group is given in Table -8)

Table - 8 Lobular Type distributions in relation to parity

Age group	Lobular type I		Lobular type II		Lobular type III	
	Parous	Nulliparous	Parous	Nulliparous	Parous	Nulliparous
11-20	-	4	1	1	1	-
21-30	1	1	1	-	3	-
31-40	-	1	1	1	5	-
41-50	1	-	3	-	4	-
51-60	6	-	1	-	-	-
	8	6	7	2	13	0

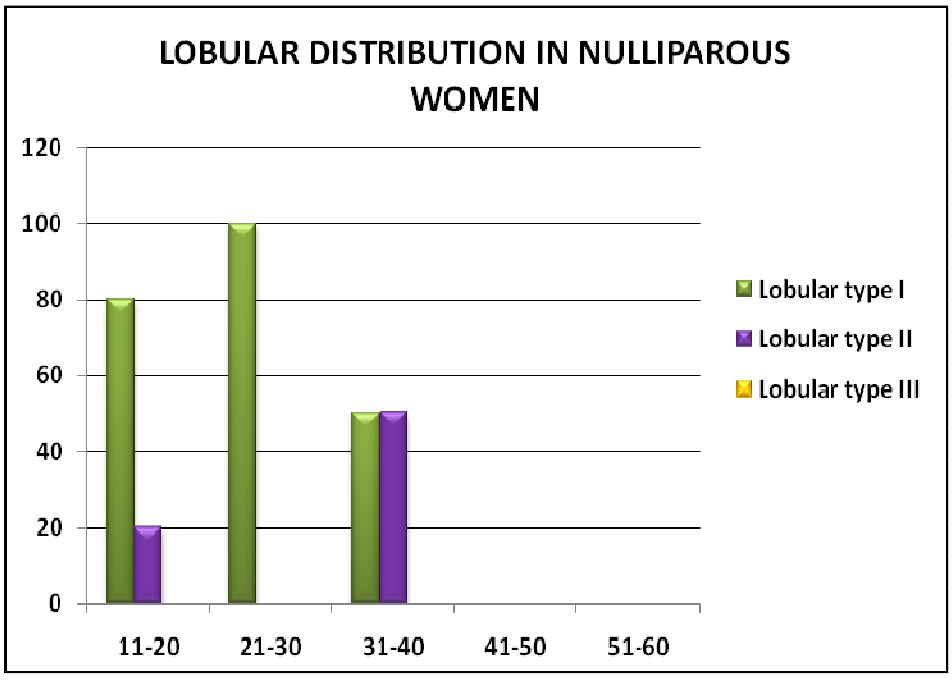
Using the above table, each sample was analyzed and plotted against each age group.

In Nulliparous Women

Table - 9 Lobular Type distribution in Nulliparous Women

Age group	Total samples	Lobular type I	%	Lobular type II	%	Lobular type III	%
11-20	5	**4**	**80**	**1**	**20**	-	0
21-30	1	**1**	**100**	-	0	-	0
31-40	2	**1**	**50**	**1**	**50**	-	0
41-50	-	-	0	-	0	-	0
51-60	-	-	0	-	0	-	0
Total	**8**	**6**	0	**2**	0	0	0

Chart 3



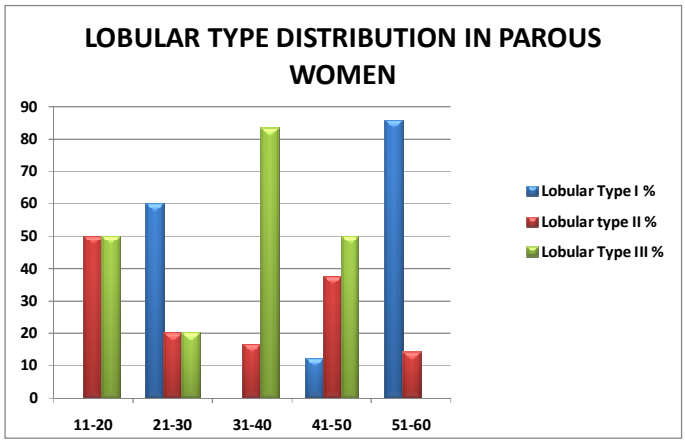
There were total of 8 samples. Out of these 8 samples analyzed, 5 were in the age group 11-21 years. Among these, 4 samples of nulliparous women showed Lobular Type I (80%) and one sample showed Lobular Type II (20%).The age group 21-30 years included 6 samples out of which only one sample was from nulliparous women and the lobular architecture was Type I. The age group 31-40 years included 2 samples from nulliparous women, of which each one showed Lobular Type I and II respectively. Age group 41 to 50 and 51 to 60 did not have any samples.

In Parous women:

Table - 10 Lobular Type distributions in Parous Women

Age group	Total samples	Lobular type I	%	Lobular type II	%	Lobular type III	%
11-20	2	-	0	**1**	**50**	**1**	**50**
21-30	5	**1**	**60**	**1**	**20**	**3**	**20**
31-40	6	-	0	**1**	**16.7**	**5**	**83.3**
41-50	8	**1**	**12.5**	**3**	**37.5**	**4**	**50**
51-60	7	**6**	**85.7**	**1**	**14.2**	-	0
Total	**28**	**8**		**7**		**13**	

Chart - 4



In the age group 11-20 years, total of 2 samples were analyzed. Of this one sample showed Type II (50%) and the other showed Type III (50%) lobular structure. Lobular Type I was not seen in this age group.

In the age group 21-30 years, total of 5 samples were analyzed. Out of these 5 samples, three samples showed Type III (60%) lobular structure. Type II and Type I were seen in one sample each (20%).

In the age group 31-40 years, a total of 6 samples were analyzed. Five samples (83.3%) showed Type III lobules and one sample showed Type II lobule (16.7%). Type I lobule was not seen in this age group.

In the age group 41-50 years, out of 8 samples, four showed Type III (50%), three samples showed Type II (37.5%) and one sample showed Type I (12.5).

In the age group of 51-60 years, six out of seven samples showed Type I lobular architecture (85.7%) and one samples showed Type II lobular architecture (14.3%). No Type III lobule was seen.

CHANGES IN THE NORMAL BREAST TISSUE IN RELATION TO PHASE OF MENSTRUAL CYCLE

To analyze the relation of menstrual cycle with the changes in normal breast tissue in reproductive age group, 11 samples from women between age group 18-42 years with regular menstrual cycle and known last menstrual period (LMP) were taken.

Based on morphological criteria of Vogel *et,*[10] when chronological phase was compared to the morphological phases of 11 samples, the following observation was plotted. Weightage was given for myoepithelial cell changes and intralobular stromal changes for assigning the morphological phase.

The menstrual cycle was standardized to 28 days and phases were assigned as described in Materials and Methods and each criterion were plotted as given in Table -11

Table – 11a Morphological changes in the intra lobular stroma

Sample No:	Chronological phase #	Intralobular Stroma	
		Dense/Loose	Cellularity
5	III	Loose	+
6	II	Dense	+++
7	IV	Loose	+
9	III	Dense	+
10	IV	Loose	+
11	IV	Loose	+
12	I	Dense	++
13	IV	Loose	+
20	IV	Loose	+
21	III	Loose	++
23	IV	Loose	+

Chronological phase: I (3-7days); phase II (8-14days);

phase III (15-20days); phase IV (21-27days); phase V (28-2days)

Out of 11 samples studied, the intralobular stroma was dense, resembling phase I and II of chronological phase, in 3 samples. Two of these samples showed mild to moderate edema. (Photo 5-Loose stroma)

Table – 11b Morphological changes in the acini

Sample no:	Chronological phase	Acini	
		Secretion	Lumen
5	III	+	Open
6	II	-	Closed
7	IV	-	Distended
9	III	-	Closed
10	IV	++	Distended
11	IV	++	Distended
12	I	-	Closed
13	IV	+	Open
20	IV	++	Distended
21	III	-	Closed
23	IV	++	Distended

Out of 11 samples studied, the lumen was closed in 4 samples and showed no active secretion in all of the four samples. Lumen was open with some secretion in 2 samples and showed active secretion with distention of lumen in 5 samples. (Photo 6 - Dilated lumen with secretion)

Table – 11c Morphological changes in the epithelial layer

Sample no:	Chronological phase	Epithelium	
		Stratification	Myoepithelial layer
5	III	+	Conspicuous
6	II	-	Not conspicuous
7	IV	+	Conspicuous
9	III	-	Not conspicuous
10	IV	+	Conspicuous
11	IV	+	Conspicuous
12	I	-	Not conspicuous
13	IV	+	Conspicuous
20	IV	+	Conspicuous
21	III	-	Conspicuous
23	IV	+	Conspicuous

Out of 11 samples studied, epithelial stratification was present in 7 cases and absent in 4 cases. Myoepithelial cells were conspicuous in 8 samples and inconspicuous in 3 samples. (Photo7 – Epithelial stratification)

Table -11d Morphological changes in the myoepithelium

Sample no:	Chronological Phase # (day of cycle)	Myoepithelial vacuolation
5	III	+*
6	II	-
7	IV	++*
9	III	+
10	IV	++
11	IV	++
12	I	-
13	IV	+
20	IV	++
21	III	+
23	IV	++

*(+) - denotes <30% of ductules shows vacuolation, (++) - denotes >70% of ductules shows vacuolation. # Chronological phase: I (3-7days);

phase II (8-14days); phase III (15-20days); phase IV (21-27days); phase V (28-2days)

Prominent vacuolation in myoepithelial cell layer (>70% of ductules) was seen in 6 samples, was absent in 2 samples. 3 samples showed myoepithelial vacuolation in < 30% of ductules. (Photo 8- Myoepithelial vacuolation)

Table - 11e Morphological changes in the nucleus

Sample no:	Chronological phase#	Mitosis
5	III	Absent
	II	Absent
7	IV	Absent
9	III	Absent
10	IV	Absent
11	IV	Absent
12	I	Absent
13	IV	Absent
20	IV	Absent
21	III	Absent
23	IV	Absent

Chronological phase: I (3-7days); phase II (8-14days); phase III (15-20days); phase IV(21-27days); phase V (28-2days).

In the 11 samples studied, none of the samples showed mitotic activity.

Table - 12 Comparing the morphological phase with chronological phase

Sample no:	Chronological phase	Morphological phase
5	III	III
6	II	II
7	IV	IV
9	**III**	**II**
10	IV	IV
11	IV	IV
12	**I**	**II**
13	IV	IV
20	IV	IV
21	**III**	**II**
23	IV	IV

Of the 11 samples, 8 **(72.7%)** samples correlated with the chronological phase of menstrual cycle and 3 samples belonged to adjacent phase of menstrual cycle.

CHANGES IN THE MYOEPITHELIAL CELL WITH AGE & LOBULAR TYPE

Immunohistochemistry staining was done for 4 slides, in the age 20 years (nulliparous), 21 years (parous), 35 years (parous), and 55 years (postmenopausal) women, with S100 marker to demonstrate the proportion of myoepithelial cells each of these slides. Observations were made about the staining pattern, regular or irregular; and intensity of staining, determined by degree of staining described as 1+ (mild) to 2+ (moderate).

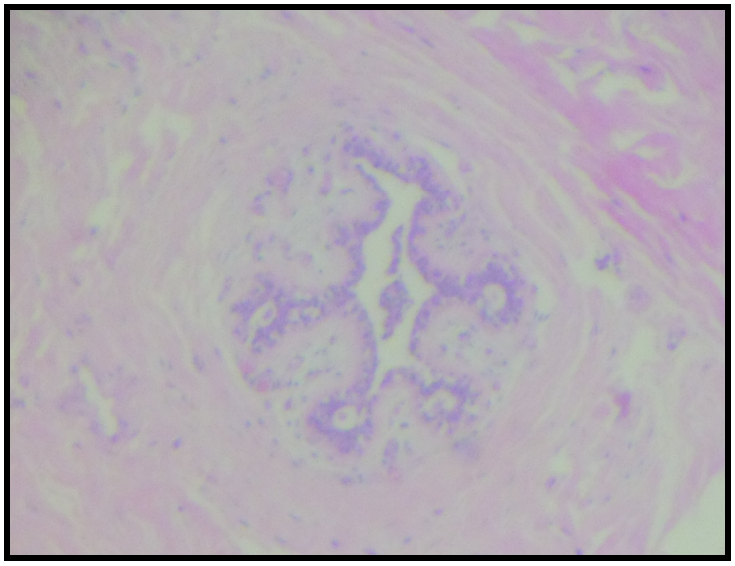
The sample no: 16, aged 20 years(nulliparous), with Type I lobular pattern showed, regular staining pattern with intensity of staining of 1+.(Photo 9)

The sample no: 9, aged 21 years(parous), with Type II lobular pattern showed, regular staining pattern with intensity of staining of 2+.(Photo - 10)

The sample no: 27, aged 35 years (parous), with Type III lobular pattern showed, regular staining pattern with intensity of staining of 2+. (Photo - 11)

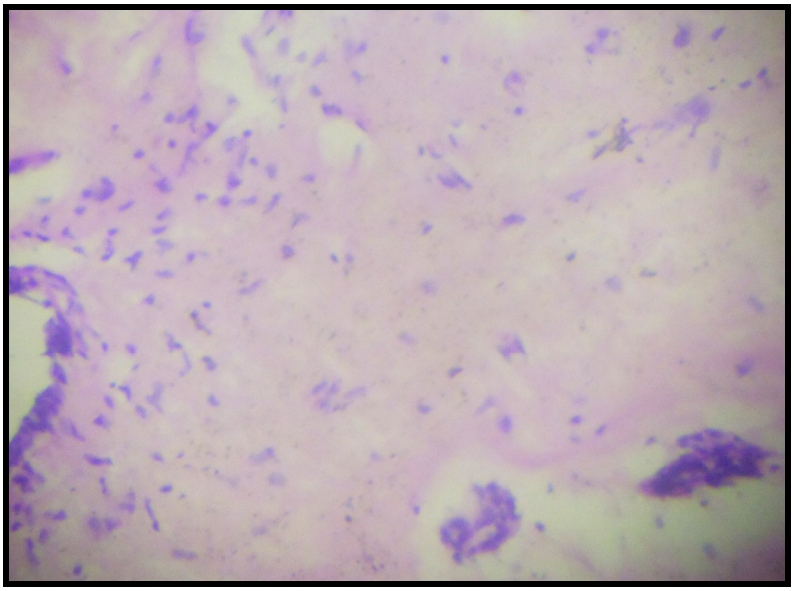
The sample no: 32, aged 55 years(postmenopausal) with Type I lobular pattern showed, irregular staining pattern with intensity of staining of 1+. (Photo - 12)

Photo -1 Lobular Type - I with Terminal End Bud - TEB



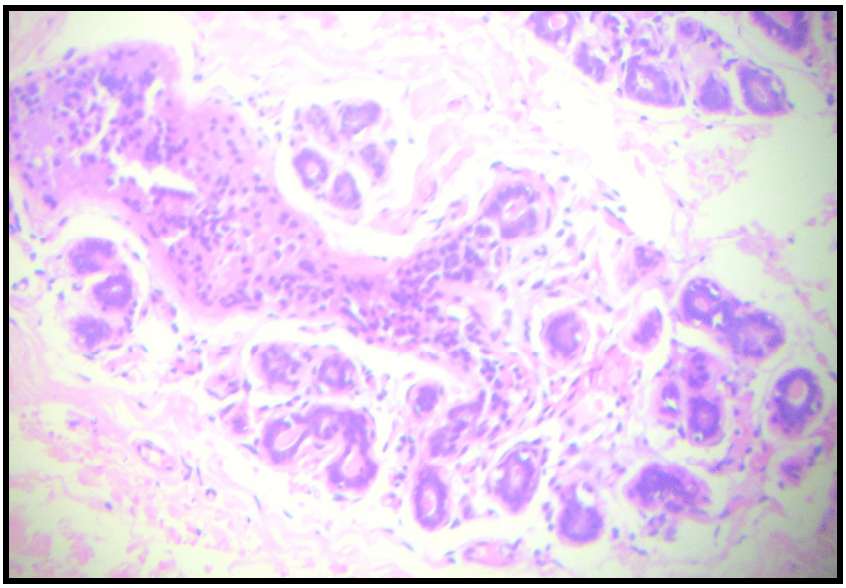
S.No:16 Low power view

Photo - 2 Lobular Type - I Postmenopausal



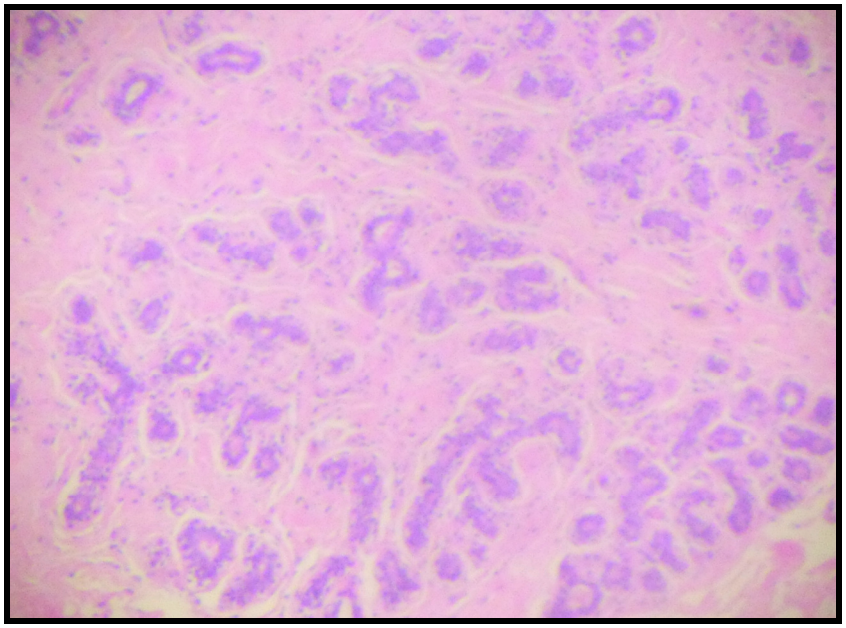
S.No:16 Low power view

Photo - 3 **Lobular Type - II**



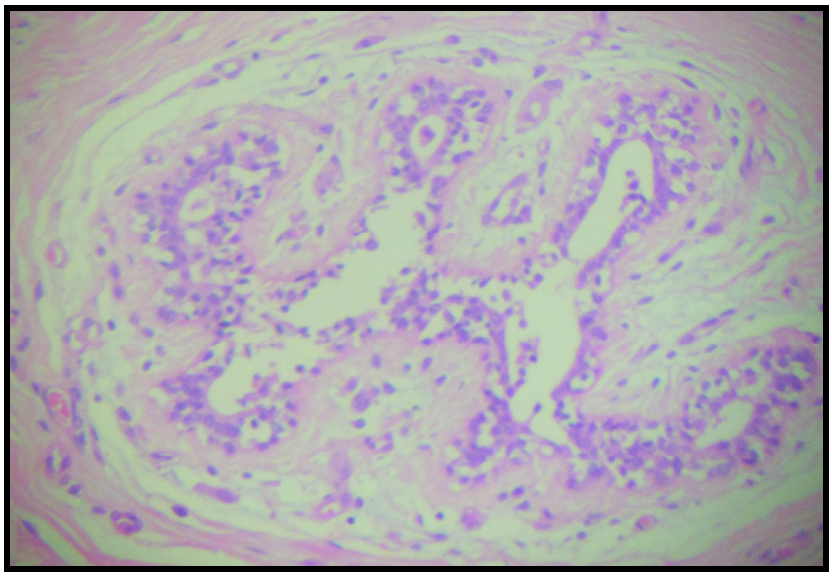
S.No:9 Low power view

Photo - 4 **Lobular Type - III**



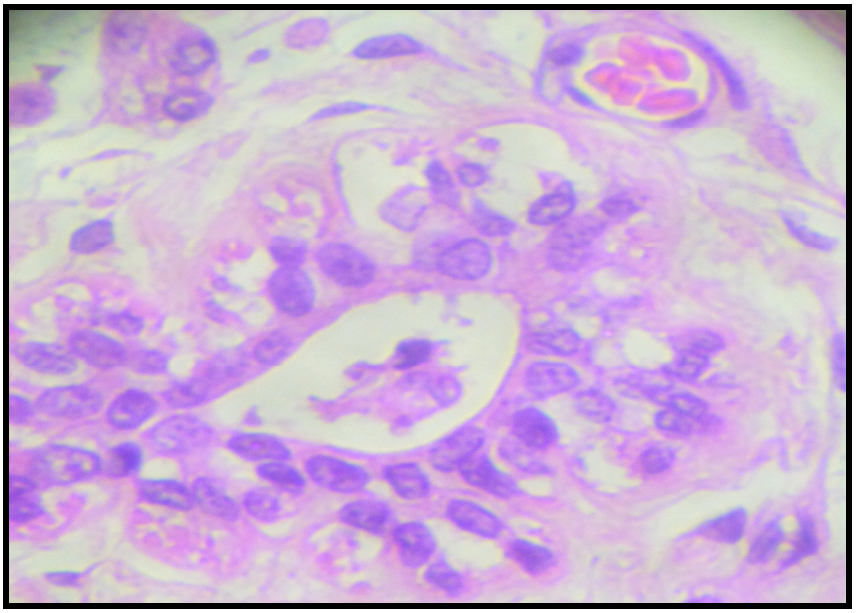
S.No:27 Low power view

Photo - 5 **Loose intra lobular stroma**



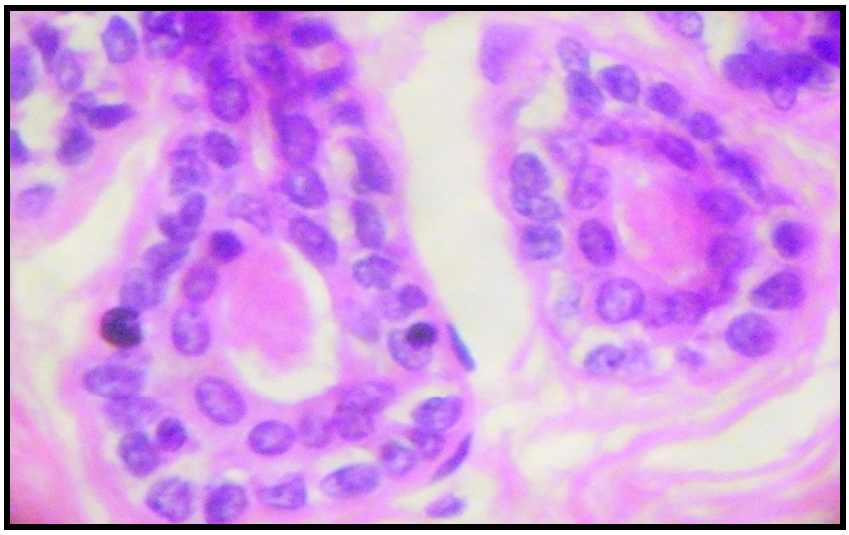
S.No:16 Low power view

Photo - 6 **Dilated lumen with secretion**



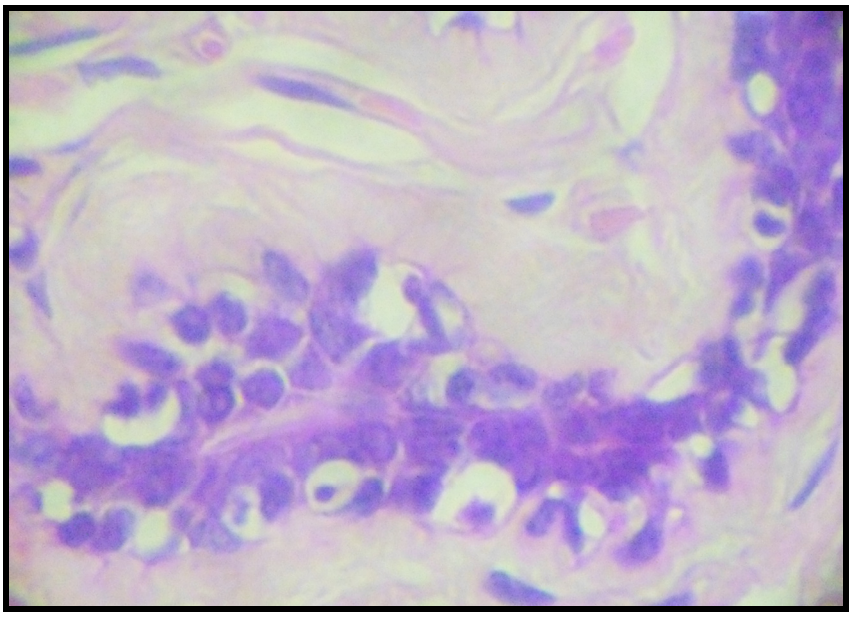
s.No:23 High power view

Photo -7 Epithelial stratification



S.No:27 High power view

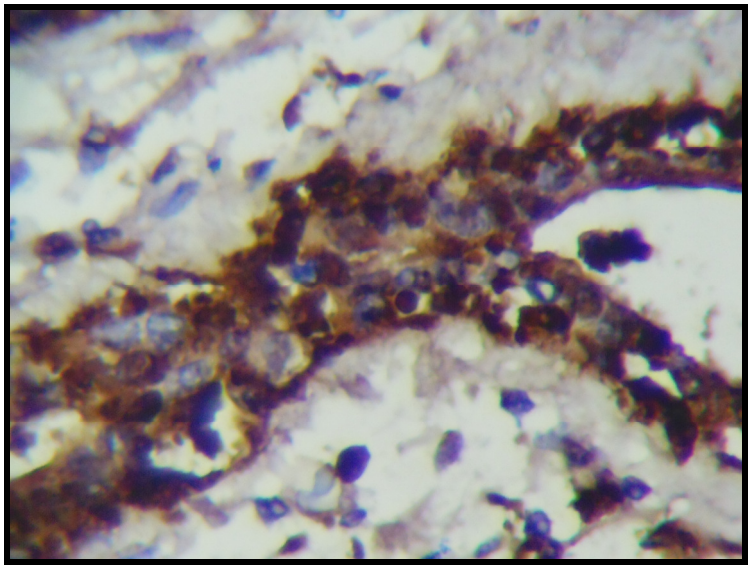
Photo - 8 Myoepithelial vacuolation



S.No:23 High power view

Photo - 9 IHC Myoepithelium in 20 year s of age

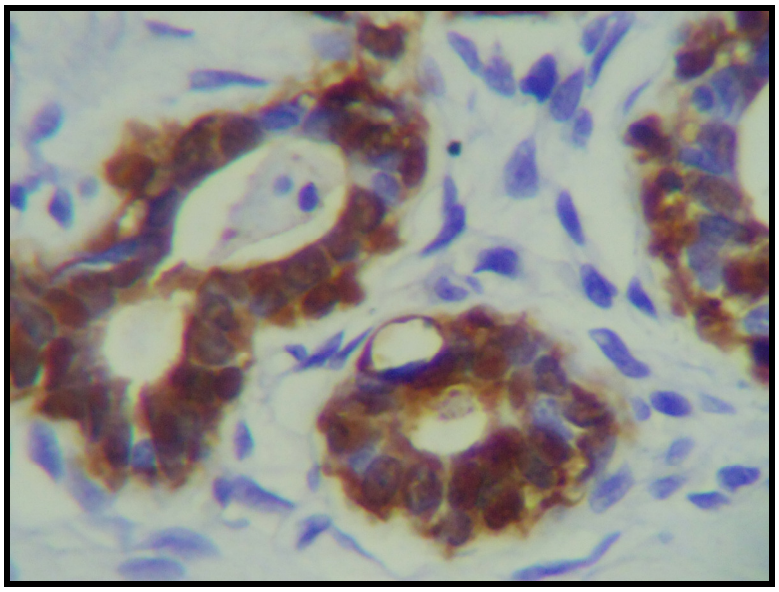
(Nulliparous women, Lobular Type I)



S.No:16 High power view

Photo - 10 IHC Myoepithelium in 21 year s of age

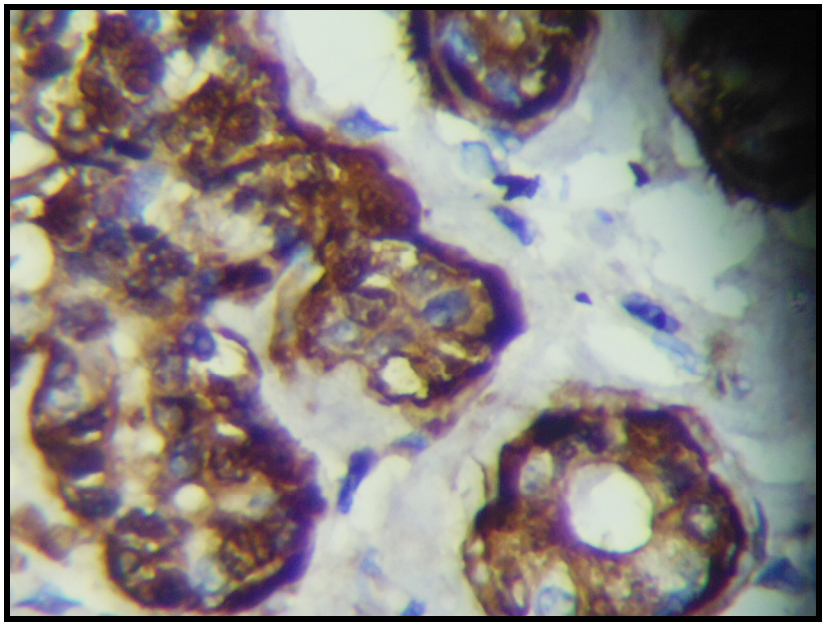
(Parous women, Lobular Type II)



S.No: 9 High power view

Photo - 11 IHC Myoepithelium in 35 years of age

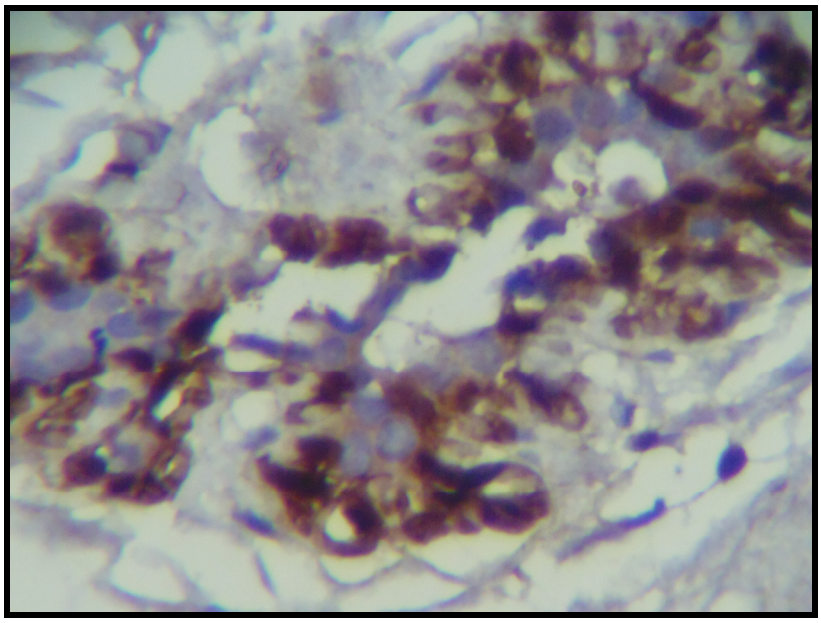
(Parous women, Lobular Type III)



S.No:27 High power view

Photo - 12 IHC Myoepithelium in 55 year s of age

(Parous women, Lobular Type I)



S.No: 32 High power view

DISCUSSION

In the present study, age and parity influences the histological structure of the mammary gland tissue. The present study showed that in the women of age < 20 years, the most common type of lobule was Lobular type I (71.4%), which decreases greatly After 3rd decade to <15%. Lobular type I showed increase in the fifth decade (85.7%) to become the predominant type of structure.

In the study of Russo et al, women < 20years of age showed all the three types of Lobular structure in equal proportion. After 23 years, Lobular type I showed reduction in number and in the 4th decade increases to become the predominant type of lobule (70%).

Hence, in the present study is not in concordance with the study of Russo et al regarding Lobular type I as the predominant type of lobule.

Lobular type II in the present study showed no significant alteration in the proportion (14% to 16%) except in the 3rd and 4th decades, where the value slightly increased (25% to 37%).

Russo et al, in his study, found no significant alteration in the Lobular type II proportion, which remained unchanged until 5^{th} decade. After 5^{th} decade, it decreased to < 5%.

Hence, in the present study is in concordance with the study of Russo et al regarding Lobular type II.

In the present study, Lobular type III was seen as the predominant type of lobular structure, in the age between 21 to 50 years. This type was completely absent after 5th decade.

In the study of Russo et al, Lobular type III increased after 23 years of age and reaches maximum of 70% by 3rd decade of life. This proportion was maintained till midforties. The vale decreased significantly after 5^{th} decade to around 15%.

Hence, in the present study is in concordance with the study of Russo et al regarding Lobular type III.

In the present study of breast tissue in relation to age and parity, among the 28 samples from parous women, Lobular type III was seen in 13 samples, in the age of 21to 50 years. Lobular type II was the second common type, which showed slight increase in the midforties. Lobular type I was the least common type, which becomes the predominant type after 50 years.

Of the 8 samples from the nulliparous women, the predominant architecture was Lobular type I (75 %), maximally seen in, 20 years of age. The second common type of lobular structure was Lobular type II (25 %), seen between 20 to 30 years of age. Lobular type III was not seen in any of the 8 samples studied.

From the above observations, it is evident that nulliparous women have more of undifferentiated lobules (Lobular type I) and lack fully differentiated lobules, which is seen only after full term pregnancy. In the parous women, as the lobular differentiation is complete during pregnancy, they exhibit maximum percentage of fully differentiated lobules (Lobular type III).

In the parous women, Lobular type II showed slight increase in the midforties and this may be due to involution of Type III lobular structure. Whereas, Lobular type II in nulliparous women may be due to differentiation of Type I lobular structure. Since there was no nulliparous samples were included in the age groups of 41 to 60 years, the lobular type could not be analyzed.

Although ductal carcinoma arises in the Lobular type I or TDLU, the epidemiological observation points out the increased incidence of breast cancer among nulliparous women than parous women.

This may be due to the difference in the susceptibility of Lobular type I in both the groups to carcinogenesis. The presence of Lobular type I has been interpreted as failure of mammary gland to respond to the hormonal influence during pregnancy and lactation. Hence, parous women may have Lobular type I due to both regression and failure of differentiation.

The difference in the sensitivity of Lobular Type I to carcinogenesis could not be answered in the present time. The theory that type I was due to regression of fully differentiated lobule was supported by the observation of the presence of hyalinization of intra lobular stroma in Lobular type I of parous women and not in nulliparous women.

In parallel studies, cell kinetics of all the three types (Lobular type I, II, III), were different and also exhibit difference in the susceptibility to carcinogenesis. Lobular type I expressed malignant features on treatment with carcinogens, but not by Lobular type III.

As it has been reviewed, the incidence of atypical lobular hyperplasia and Carcinoma insitu (CIS) originates in Lobular Type II, and it is possible that the incidence also decreases due to reduction in the number of Type II lobule.

The stimulus of pregnancy or exogenous hormones differentiates the terminal duct lobular unit to fully lobular architecture. The persistence of this protection even after the termination of pregnancy or the hormonal treatment indicate permanent modification of the morphological architecture of the mammary gland, even though with age, the lobule may regress to more primitive type.

Studies on factors increasing the breast cancer risk states that the number of non-reproductive ovular cycle increases the risk of carcinoma. i.e. the more the number of cell division, that prepares the breast for more proliferation and differentiation during pregnancy, greater will be the risk. The reduction in the total number of menstrual cycle alone is not sufficient for the protection, but more likely to be due to change in the differentiation status of the breast. In this study, only the degree of differentiation of lobular structure was observed and hence other factors could not be discussed. However, parous women may contain different balance of stem cell and differentiated proliferative cells due to the increase in the number of differentiated cells as compared to stem cells, which may play major role in carcinogenesis of breast tissue.

In the present study, the morphological criteria of Vogel *et al* were slightly modified to correlate the date of menstrual cycle with the surgical specimen of breast tissue.

The morphological dating of breast tissue is important to time the surgery during the proliferative phase (luteal phase), as stated by some studies. The present study has attempted to determine the proliferative phase of tissue. Even though no single criterion established any particular morphological phase, the proportion of vacuolation in myoepithelium; luminal secretion; and changes in intra lobular stroma were found useful; in the characterization of the morphological phase of menstrual phase. In this study, none of the 11 samples showed mitotic activity.

In the present study, the morphologically “normal” lobule within the diseased breast tissue was used for dating the breast tissue. The agreement between the morphological phase and the chronological date was not absolute. Only 8 out of 11 samples agreed with the date of the cycle. This disagreement could be due to either morphological or chronological factors. The chronological date, although gold standard, fails to recognize events like lack of ovulation and varying cycle length. In the standardization of varying cycle length to 28 day cycle, the formula was based on the assumption that the luteal phase is of fixed duration, even though variability in this phase is documented in earlier studies. These limitations stress the importance of evaluation of the morphological phase of breast tissue with that of menstrual cycle.

Immunohistochemical study of the 4 slides in the present study, the slide of 20 year nulliparous women and 21 year old parous women showed regular staining pattern, but the intensity was more (2+) in parous than the nulliparous women(1+). The slide of 35 years parous women showed the same regular pattern and intensity as that of 21 years parous women. The postmenopausal age sample slide showed decrease in the intensity with irregular pattern of staining. These observations agree with the study of Milanese *et al*[85] and Hutson *et al*[86] in that the degree of staining intensity and pattern decreases with age.

When IHC slides were compared with the types of lobule, the staining pattern of Type I to Type III lobule was regular in all 3 types. The degree of staining intensity was more in Type II & III than Type I. But, the pattern and intensity of premenopausal age (20 years) Type I was regular and as compared to the postmenopausal age (55years) Type I, which showed irregular staining pattern.

CONCLUSION

The observation made in this study showed that lobular differentiation progresses from Type I to Type III as age increases and shows regressive changes from Type III to Type I after 50 years of age. This progressive differentiation is seen in parous women only. Regressive change is seen in both parous and nulliparous women.

In nulliparous women, the breast tissue show maximal differentiation from Type I to Type II Only. Type III differentiation was not seen. This emphasizes that differentiation is not complete due to lack action of pregnancy induced hormonal changes. Lobular type II did not show much change in the percentage both in nulliparous and parous women.

Observation of normal breast tissue during different phases of menstrual cycle for assigning morphological phase to date of the cycle (chronological phase) showed only 72.7% of concordance.

Immunohistochemical study for demonstrating myoepithelial changes with age showed decreased staining intensity and irregular pattern as age advances. This may be due to the changes in the cell morphology of myoepithelium with age.

As the above observations were made in a very limited sample size statistical significance could not be determined. This precluded quantitative study of breast tissue in relation to age, parity and menstrual cycle and hence attempt was made for qualitative study.

The clinical implication of this study is to provide the basis for more accurate interpretation of the histomorphology of normal breast parenchyma in relation to age, parity and to compare the morphological phase with the chronological date of menstrual cycle, in the surgical biopsy tissue and mastectomy specimen.

On knowing that the lobular Type I, in both nulliparous and parous women caries more risk for developing carcinoma as compared to Type III.This is more so in the case of nulliparous than parous women. Hence these patients can be followed up frequently to prevent development of malignancy or diagnose it early. The morphological phase assignment helps in the timing of breast surgery during the luteal phase.

The IHC study of the pattern and intensity by myoepithelium may help in identifying the normal morphological changes with age, as they are important for determining the invasiveness of the carcinoma.

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BIBILIOGRAPHY

1. Greenlee RT, Murray T, Bolden S, Wingo PA (2000) Cancer statistics, 2000. CA Cancer J Clin 50:7–33.

2. Kelsey, J. L., Gammon, M. D., and John, E. M. Reproductive factors and breast cancer. Epidemiologic Rev., 15: 36-47, 1993.

3. Yuan, J-M., Yu, M. C., Ross, R. K., et al. Risk factors for breast cancer in Chinese women in Shanghai. Cancer Res., 48: 1949-1953, 1988.

4. Ewertz, M., and Duffy, S. W. Risk of breast cancer in relation to reproductive factors in Denmark. Br J Cancer, 58: 99-1 04, 1988.

5. Ewertz, M., Duffy, S. W., Adami, H.O., et al. Age first birth, parity and of breast cancer: a met analysis of 8 studies from the Nordic countries. Int. J. Cancer, 46: 597-603, 1990.

6. Lund, E. Breast cancer mortality and the change in fertility risk factors at menopause: a prospective study of 800,000 married Norwegian women. Epidemiology,2: 285-288, 1991.

7. Russo, J., and Russo, I. H. Biological and molecular bases of mammary carcinogenesis. Lab. Invest. 57: 1 1 2-1 37, 1987.

8. Russo, J., and Russo, I. H. Is differentiation the answer in breast cancer prevention? IRCS Med. Sci., 10: 935-941, 1982.

9. Russo, J., Tay. L. K., and Russo, l. H. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res. Treat., 2: 5-73, 1982.

10. Vogel, P.M., Georgiade, N.G., Fetter, B.J., VOGEL, F.S. &McCarty, K.S. Jr (1981). The correlation of histological changes in the human breast with the menstrual cycle. Am. J. Pathol., 104, 23.

11. Longacre T, Bartow S. A correlative morphologic study of human breast and endometrium in the menstrual cycle. Am J Surg Pathol 1986; 10: 382–393.

12. Going J, Anderson T, Battersby S, MacIntyre C. Proliferative and secretory activity in human breast during natural and artificial menstrual cycles. Am J Pathol 1988; 130: 193-204.

13. Ferguson, D. J. P., and Anderson, T. J. Morphological evaluation of cell turnover in relation to the menstrual cycle in the resting human breast. Br. J.Cancer, 44: 177-181, 1981.

14. Anderson, T.J., Ferguson, D.J.P. & Raob, G.M. (1982). Cell turnover in the 'resting' human breast: Influence of parity, contraceptive pill, age and laterality. Br. J. Cancer, 46, 376.

15. Mauvais-Jarvis, P., Kuttenn, F. & Gourpel, A. (1986). Antiestrogen action of progesterone in breast tissue. Breast Cancer Res. Treatment, 8: 179.

16. Dawson, E. K. A histological study of the normal mamma in relation to tumor growth. I. Early developrnental maturity. Edinb. Med. J., 41: 653-682.1934.

17. Wellings, S. R., Jensen, H. M., and Marcurn, R. G. An atlas of subgross pathology of 1 6 human breasts with special reference to possible precancerous lesions. J. Nat I. Cancer Inst., 55: 231-275, 1975.

18. Geschickter, C. F. Normal development and functional changes in the mammary gland. In: C. F. Geschickter, (ed), Diseases of the Breast: Diagnosis, Pathology, Treatment, p 3-41. Philadelphia: Lippincott, 1945.

19. Potten C, Watson R, Williams G, Tickle S, Roberts S, Harris M, et al. The effect of age and menstrual cycle upon proliferative activity of the normal human breast. Br J Cancer. 1988; 58: 163–170.

20. Stockard, Mary (2005) Raising Orphaned Baby Opossums. Alabama Wildlife Center.

21. Julie Ann Mayer, John Foley, Damon De La Cruz, Cheng-Ming Chuong, and Randall Widelitz" ("november 2008"). "Conversion of the Nipple to Hair-Bearing Epithelia by Lowering Bone Morphogenetic Protein Pathway Activity at the Dermal-Epidermal Interface.

22. "BBC News – The goats with spider genes and silk in their milk". bbc.co.uk. 17 January 2012. Retrieved on 26 April 2012.

23. Oftedal, OT (2002). "The mammary gland and its origin during synapsid evolution". Journal of Mammary Gland Biology and Neoplasia 7 (3): 225–52.

24. Vorbach, C.; Capecchi, M. R.; Penninger, J. M. (2006). "Evolution of the mammary gland from the innate immune system?". Bio Essays 28 (6): 606–616.

25.Asma Javed and Aida Lteif. Development of the Human Breast. Semin Plast Surg. Feb 2013; 27(1): 5–12.

26. Collins LC, Schnitt SJ. Normal breast. In: Mills SE, ed. Histology for Pathologists. Philadelphia:Lippincott Williams & Wilkins; 2007:57–71.

27. Rosen PP. Rosen's Breast Pathology. Philadelphia: Lippincott Williams & Wilkins; 2009.

28. Anbazhagan R, Bartek J, Monaghan P, et al. Growth and development of the human infant breast. Am J Anat.1991;192(4):407–417.

29 Jose Russo, Irma H. Russo. Development of the human breast. Maturitas Volume 49, Issue 1, Pages 2–15, September 24, 2004.

30. The development of the reproductive system. In: Tanner JM, editor. Growth at adolescence. Oxford, UK: Blackwell Scientific; 1962. p. 28–39.

31. Development of the female breast. In: Vorherr H, editor. The breast. New York: Academic Press; 1974. p. 1–18.

32 Kristen A Atkins.,Christina.S.Kong. Practical Breast Pathology., A Diagnostic approach. Jonathan,M., Kitayama,M.D., and Teri,A., Logacre,M.D. In: The Normal Breast.p1-14. Elsevier Saunders.

33. Schnitt SJ, Millis RR, Hanby AM, et al. Breast. In: Mills SE, Carter D, Greenson JK, et al., eds. Sternberg's Diagnostic Surgical Pathology. Philadelphia: Lippinott Williams & Wilkins; 2010:323–398.

34.Sarah E. Pinder, Ann Marie mulligan. Breast Pathology. James. J. Going, In: normal Breast.edn.2.

35. Russo J, Rivera R, Russo IH. Influence of age and parity on the development of the human breast. Breast Cancer ResTreat 1992;23(3): 211–218.

36. Russo J, Romero AL, Russo IH. Architectural pattern of the normal and cancerous breast under the influence of parity.Cancer Epidemiol Biomarkers Prev 1994;3(3):219–224.

37. Umlas J. Gynecomastia-like lesions in the female breast. Arch Pathol Lab Med. 2000;124: 844–847.

38. Nielsen TO, Hsu FD, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004;10: 5367–5374.

39. Battersby S, Robertson BJ, Anderson TJ, et al. Influence of menstrual cycle, parity and oral contraceptive use on steroid hormone receptors in normal breast. Br J Cancer. 1992; 65: 601–607.

40. Kohler S, Rouse RV, Smoller BR. The differential diagnosis of pagetoid cells in the epidermis. Mod Pathol. 1998;11:79–92.

41. Darryl Carter. Interpretation of Breast biopsies.In:ch-1 Anatomy. Edn 4 Philadelphia : Lippincott Williams & Wilkins, cop. 2003.p 1-16.

42. Russo J, Gusterson BA, Rogers AE, Russo IH, Welling SSR, Van Zwieten M J: Comparative study of human and rat mammary tumorigenesis. Lab Invest 62: 244.-278, 1990.

43. Russo IH, Russo J: Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz (a) anthracene. J Natl Cancer lnst 6l: t439-1449, 1978.

44. Ingleby, H., Gersohn-Cohen, J. The normal breast . In: H. Ingleby and J. Gersohn-Cohen (eds.), Comparative Anatomy, Pathology and Roentgenology of the Breast, pp. 3-1 1 9. Philadelphia: Univ. Penn Press, 1960.

45. McCarty, K.S., Jr, Miller, L.S., Cox, E.B., Konrath, J. &McCarty, K.S. Sr(1985). Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch. Pathol. Lab. Med., 109, 716.

46. King, R.J.B. (1990). Role of estrogen and progestin in human mammary carcinogenesis. In Endocrine Therapy of Breast CancerIV, Goldhirsch, A. (ed.), p. 3. Springer-Verlag: Berlin.

47. Lessey, B.A., Killam, A.P., Metzger, D.A., Haney, A.F., Greene,G.L. & McCarty, K.S. JR (1988). Immunohistochemical analysis of human

uterine estrogen and progesterone receptors throughout the menstrual cycle. J. Clin. Endocrinol. Metab., 67, 334.

48. Joyeux, C., Chalbos, D. & Rochefort, H. (1990). Effects of progestins and menstrual cycle on fatty acid synthetase and progesterone receptor in human mammary glands. J. Clin. Endocrinol. Metab., 70, 1438.

49. Press, M.F., Nousek-Goebl, N.A., King, W.J., Herbst, A.L. &Greene, G.L. (1984). Immunohistochemical assessment of estrogen receptor distribution in the human endometrium throughout the menstrual cycle. Lab. Invest., 51, 495.

50. Hutson SW, Cowen PN, Bird CC: Morphologic studies of age related changes in normal human breast and their significance to evolution of mammary cancer. J Clin Pathol 38: 281-287, 1985 .

51. Rosenberg A: Uber Menstruelle durch das Corpus luteum bedingte Mammaveranderungen. Frankfurt Z. Pathol 1922, 27:466-506.

52. Diekmann H: Uber die Histologie der Brustdruse bei gestortem und ungestortem Menstruationsablauf. VirchowsArch (Pathol Anat) 1925, 56:321-356.

53. Moskowitz L: Uber den monatlichen Zyklus der Brustdru. Arch Klin Chir 1926, 142:374-418.

54. Centeno J: Uber die cyklischen Veranderungen der weiblichen Brustdruse: Beitrage zur pathologischen Anatomie und zur allgemeinen Pathologie 1927, 78: 594-617.

55. Polano 0: Untersuchungen uber die zyklischen Veranderungen der weiblichen Brust wahrend der Geschlechtsreife. Z Gerburtshilfe Gynaekol 1924, 87: 363-372.

56. Speert H. Rhesus monkey-A preliminary report. Surg Gynecol Obstet .941, 73:388-390.

57. Ozzello L, Speer FD: The mucopolysaccharides in the normal and diseased breast. Am J Pathol 1958, 34:993-1099.

58. 26. Zeppa R: Vascular response of the breast to estrogen. JClin Endocrinol Metab 1969, 29:695-700.

59 Bassler R, Schafer A: Elektronen mikroskopische Cytomorphologie der Gynakomastie. Virchows Arch (Pathol Anat) 1969, 348:356-373.

60. Macmahon, B., Cole, P., Lin, T.M. & 6 others (1970). Age at first birth and breast cancer risk. Bull WHO, 43, 209.

61. Haagensen CD: Diseases of the Breast. Philadelphia, W. B. Saunders Company, 1971, pp57-62.

62. Radnor CJP: Myoepithelial cell differentiation in rat mammary glands. J Anat 1972, 3:381-398.

63. Fanger H, Ree HJ: Cyclic changes of human mammary gland epithelium in relation to the menstrual cycle-an ultrastructural study. Cancer 1974, 34:571-585.

64. Meyer JS: Cell proliferation in normal human breast ducts, fibroadenomas, and other ductal hyperplasias measured by nuclear labelling with tritiated thymidine.Hum Pathol 1977, 8:67-81.

65. Pike, M.C., Henderson, B.E., Krailo, M.D., Duke, A. & Roy, S. (1983). Breast cancer in young women and use of oral contraceptives: Possible modifying effect of formulation and age at use.Lancet, ii, 926.

66. McManus, M.J. & Welsche, C.W. (1984). The effect of estrogen, progesterone, thyroxine, and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. Cancer, 54, 1920.

67. La Vecchia, C., Decarli, A., Di Pietro, S., Franceschi, S.,Negri, E. & Parazzini, F. (1985). Menstrual cycle patterns and the risk of breast disease. Eur. J. Cancer Clin. Oncol., 21, 417.

68. Murphy, L.J., Murphy, L.C., Stead, B., Sutherland, R.L. & Lazarus, L. (1986a). Modulation of lactogenic receptors by progestins in cultured human breast cancer cells. J. Clin. Endocrinol. Metab., 62, 280.

69. Ferguson D. An ultra-structural study of mitosis and cytokinesis in normal "resting" human breast. Cell Tissue Res 1988; 252: 581–587.

70. Potten, C.S. (1987). Possible defects in the proliferative organisation and control mechanism in psoriasis. Proc. IV Int. Psoriasis Sym. Stanford 1986. Elsevier, N.Y., p. 15.

71. Battersby, S. & Anderson, T.J. (1989). Histological changes in breast tissue that characterize recent pregnancy. Histopathology, 15, 415.

72. Jacquemier, J.D., Hassoun, J., Torrente, M. & Martin, P.M. (1990). Distribution of estrogen and progesterone receptors in healthy tissue adjacent to breast lesions at various stages -immunohistochemical study of 107 cases. Breast Cancer Res. Treat., 15, 109.

73. Joyeux, C., Chalbos, D. & Rochefort, H. (1990). Effects of progestins and menstrual cycle on fatty acid synthetase and progesterone receptor in human mammary glands. J. Clin. Endocrinol. Metab., 70, 1438.

74. Williams, G., Anderson, E., Howell, A. & 4 others (1991). Oral contraceptive (OCP) use increases proliferation and decreases estrogen receptor content of epithelial cells in the normal breast. Int. J. Cancer, 48, 206.

75. Battersby, S. & Anderson, T.J.et al. Influence of menstrual cycle, parity and oral contraceptive use on steroid hormone receptors in normal breast.Br J Cancer 4:601-607, 1992.

76. Anderson, T.J., Battersby S., King, R.J.B., McPherson, K. & Going, J.J. (1989). Oral contraceptive use influences resting breast proliferation. Human Pathol., 20, 1139.

77. Andres AC, Strange R: Apoptosis in the estrous and menstrual cycles. J Mammary Gland Biol Neoplasia 1999, 4:221-228.

78. Rathi Ramakrishnan., Seem A. Khan., and Sunil Bavade. Morphological changes in breast tissue with menstrual cycle. Mod Pathol.15 (12):1348-1356. 2002.

79.Rathi Ramakrishnan, Peter H Gann., Elizabeth L. Wiley., Kamal K. Khurana., and Seem A. Khan. Normal breast lobular architecture in breast biopsy samples from breast cancer cases and benign disease controls. Breast Cancer Res.Treat.,86: 259-268.2004.

79. Maria Alicia H Navarrete et al. Assessment of the proliferative,apoptotic and cellular renovation indices of the human mammary epithelium during the follicular and luteal phases of the menstrual cycle. Breast Cancer Research, 7:r306-R313.2005.

80. Runswick SK, O'Hare MJ, Jones L, Streuli CH, Garrod DR. Desmosomal adhesion regulates epithelial morphogenesis and cell positioning. Nat Cell Biol 2001;3:823–30.

81. Gudjonsson T, Ronnov-Jessen L, Villadsen R, Rank F, Bissell MJ, Petersen OW. Normal and tumor derived myoepithelial cells differ in their ability to interact with luminal breast epithelial cells for polarity and basement membrane deposition. J Cell Sci 2002;115:39–50.

82. 10. Sternlicht MD, Kedeshian P, Shao ZM, Safarians S, Barsky SH. The human myoepithelial cell is a natural tumor suppressor. Clin Cancer Res 1997;3:1949–58.

83. Sternlicht MD, Barsky SH. The myoepithelial defense: A host defense against cancer. Med Hypotheses 1997;48:37–46.

84. Rudland PS. Stem cells and the development of mammary cancers in experimental rats and in humans.Cancer Metastasis Rev 1987;6:55–83.

85. Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, MaloneySD, et al. Age-related lobular involution and risk of breast cancer.J Natl Cancer Inst 2006;98:1600–7.

86. Hutson SW, Cowen PN, Bird CC. Morphometric studies of age related changes in normal human breast and their significance for evolution of mammary cancer. J Clin Pathol 1985;38:281–7.

87. Well D, Yang H, Houseni M, Iruvuri S, Alzeair S, Sansovini M, et al. Agerelated structural and metabolic changes in the pelvic reproductive end organs. Semin Nucl Med 2007;37:173–84.

88. McCormack VA, Perry NM, Vinnicombe SJ, Dos Santos Silva I. Changes and tracking of mammographic density in relation to Pike's model of breast tissue aging: a UK longitudinal study. Int J Cancer. 2010; 127:452–61.

89. Howeedy AA, Virtanen I, Laitinen L, Gould NS, Koukoulis GK, GouldVE. Differential distribution of tenascin in the normal, hyperplastic, and neoplastic breast. Lab Invest 1990;63:798–806.

90. Imai S, Nishibayashi S, Takao K, Tomifuji M, Fujino T, Hasegawa M, et al. Dissociation of Oct-1 from the nuclear peripheral structure induces the cellular aging-associated collagenase gene expression. Mol Biol Cell 1997;8:2407–19.

91. Anthony L. Mescher., Junquerira's Basic Histology, Text and Atlas. Edn.12.McGraw Hill Medical. P 408-411.

92. Victor P. Eroschenko. diFiore's Atlas of Histology with Functional correlation. Edn 12. Lippincott Williams & Wilkins.p548-555.

93. Michel H. Ross., Wojuech Paulina. A Text and Atlas with Correlated Cell and Molecular Biology. Edn. 6. Lippincott Williams & Wilkins.p863-870.

94.Don W. Fawcett and Ronald P. Jensh. Bloom and Fawsett's Concise Histology. Edn.2. Arnold, a member of Holder Headlines group .p296-298.

95.Barbara Young, James Lowe, Alan Stevens, John W. Heath. Wheater's Functional Histology- A Text and Colour Atlas.edn.5. Churchill Livingstone. p386-391.

96. Vinay Kumar., Abul K. Abbas., Nelson Fausto. Robbins and Cotran's Pathological Basis of Disease.edn.8.Elsevier Saunders.p1066-1067.

Sample.no	Age		Parity		Predominant lobular type		
	years	groups	Parous	Nulliparous	Lob I	LobII	LobIII
1.	60		✓		✓		
2.	40		✓				✓
3.	50		✓		✓		
4.	38		✓				✓
5.	41		✓				✓
6.	24		✓				✓
7.	20		✓				✓
8.	60		✓		✓		
9.	21		✓			✓	
10.	40			✓		✓	
11.	30		✓				✓
12.	35			✓	✓		
13.	18			✓	✓		
14.	43		✓				✓
15.	42		✓				✓
16.	20			✓	✓		
17.	16			✓	✓		
18.	55		✓		✓		
19.	59		✓		✓		
20.	40		✓			✓	
21.	42		✓			✓	
22.	45		✓			✓	
23.	27		✓				✓
24.	44		✓			✓	
25.	41		✓				✓
26.	39		✓				✓
27.	35		✓				✓
28.	60		✓		✓		
29.	31		✓				✓
30.	15			✓	✓		
31.	19			✓	✓		
32.	57		✓		✓		
33.	19		✓			✓	
34.	22		✓		✓		
35.	24		✓		✓		
36.	52			✓	✓		

S. no	Age	Chronological phase	Epithelium		ME Vacuolation	Intralobular stroma		Acini		Mitosis
			Stratified	ME		Dense/ loose	cellularity	secretions	Lumen	
5.	41	III	+	conspicuous	+	loose	+	+	Open	Absent
6.	24	II	-	Not conspicuous	-	dense	+++	-	closed	Absent
7.	20	IV	+	conspicuous	++	Loose	+	++	Distended	Absent
9.	21	III	-	Not conspicuous	+	Dense	+	-	Closed	Absent
10.	40	IV	+	Conspicuous	++	Loose	+	++	Distended	Absent
11.	30	IV	+	Conspicuous	++	Loose	+	++	Distended	Absent
12.	35	I	-	Not conspicuous	-	Dense	++	-	Closed	Absent
13.	18	III	+	conspicuous	++	Loose	+	+	Open	Absent
20.	40	IV	+	Conspicuous	++	Loose	+	++	Distended	Absent
21.	42	III	-	Conspicuous	+	Loose	++	-	Closed	Absent
23.	27	IV	+	conspicuous	++	loose	+	++	distended	Absent